



Mayor
Dan Sullivan

Anchorage Water & Wastewater Utility

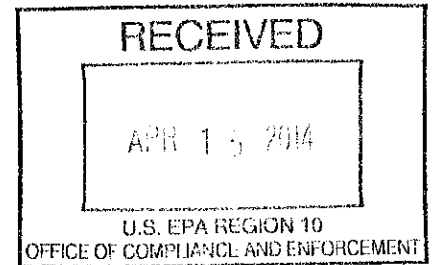
Treatment Division



Board Chair
David M. Richards

April 9th, 2014

Director, Office of Water
U.S. Environmental Protection Agency, Region 10
NPDES Compliance Unit
1200 Sixth Avenue, OW-133
Seattle, Washington 98101



**Subject: Whole Effluent Toxicity Testing Results
1st Quarter 2014
NPDES Permit No. AK-002255-1**

The John M. Asplund Water Pollution Control Facility, NPDES Permit No. AK-002255-1, requires that the results of quarterly whole effluent toxicity (WET) testing be submitted with the discharge monitoring report (DMR) for the month following the test month. The attached 3-Species WET testing report outlines test results for short-term chronic toxicity tests conducted with three species on samples collected February 17th, 19th, and 21st, 2014.

The permit requires a re-screen with three species once each year to be followed for the remainder of the next year with quarterly testing on the most sensitive species. The tests included a fertilization test using the purple sea urchin (*Strongylocentrotus purpuratus*), a bivalve larvae survival and development test using the blue mussel (*Mytilus galloprovincialis*) and a larval survival and growth test using the topsmelt (*Atherinops affinis*).

Bioassay results in this February 2014 three-species testing showed measurable toxicity in the effluent at the concentrations tested. The highest toxicity reported for this three-species WET testing was 71.4 TUc. The permit toxicity trigger of 143 TUc was not exceeded in any of the tests. Urchin gametes again appear to be the most sensitive organisms tested. For the reasons detailed in the test report, the contract laboratory recommends continuing to use the purple sea urchin as the most sensitive species for single species toxicity testing during 2014 and continuing until the three-species test is again repeated in 2015. This is consistent with results of the three-species scans conducted during 2005 through 2013 in which the urchin was the most sensitive species.

"I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of

Anchorage Water & Wastewater Utility  Clearly

3000 Arctic Boulevard • Anchorage, Alaska 99503
Phone 907-564-2799 • Fax 907-786-5681 • www.awwu.biz



FCT
4/29/14

my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations."

I can be contacted at (907) 564-2799 or <mailto:David.Persinger@awwu.biz> should you have any questions.

Sincerely,



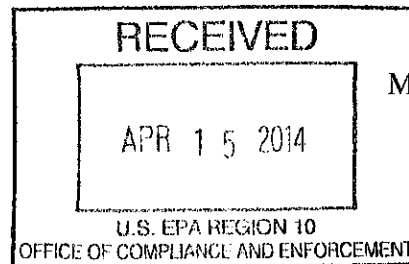
David A. Persinger, P.E.
Director, Treatment Division

Cc: Alaska Department of Environmental Conservation, Division of Water
Rob Gustafson, Water Quality Supervisor, AWWU
Jeff Axmann, Superintendent, John M. Asplund WPCF, AWWU





Gary Lawley
Kinnetic Laboratories, Inc.
1102 West 7th Avenue
Anchorage, AK 99501



March 18, 2014

Gary,

I have enclosed our report "NPDES Compliance Toxicity Testing of the Municipality of Anchorage John M. Asplund Water Pollution Control Facility Effluent: Annual Species Screening" for the effluent samples collected February 17, 19, and 21, 2014. The Anchorage NPDES permit states that "each year the permittee shall re-screen for one quarter with three species and continue to monitor for the rest of the year with the most sensitive species". Accordingly, the current species screening consisted of performing the following US EPA chronic toxicity tests:

- the echinoderm sperm fertilization test with the purple urchin, *Strongylocentrotus purpuratus*;
- the embryo-development test with the mussel, *Mytilus galloprovincialis*; and
- the 7-day survival and growth test with topsmelt, *Atherinops affinis*.

The results of this testing follow:

Effects of Anchorage Effluent on Purple Urchin Sperm Fertilization

There were significant reductions in successful fertilization at the 2.8% effluent concentration; the NOEC was 1.4% effluent, resulting in 71.4 TUc.

Effects of Anchorage Effluent on *Mytilus galloprovincialis* Embryo Development

There were no significant reductions in normal development at the any of the effluent concentrations tested; the NOEC was 2.8% effluent, resulting in 35.7 TUc.

Effects of Anchorage Effluent on *Atherinops affinis* (Topsmelt)

There were no significant reductions in survival or growth at the any of effluent concentrations tested; the NOEC was 2.8% effluent, resulting in 35.7 TUc.

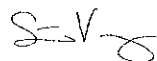
Conclusion and Recommendation

Toxicity was observed at the 2.8% effluent concentration in the purple urchin sperm fertilization test. Accordingly, and on the basis of indication from previous testing that the echinoderm (e.g., purple urchin) sperm fertilization test was the most sensitive test in the earlier screening, we recommend that the echinoderm sperm fertilization test be retained as the compliance test species for the remainder of the NPDES Permit cycle.

ICIS
4/2/14

If you have any questions regarding the performance and interpretation of these tests, please contact my colleagues Alison Briden or Dr. Scott Ogle at (707) 207-7760.

Sincerely,



Digitally signed
by Stevi Vasquez
Date: 2014.03.20
12:12:59 -07'00'

Stevi Vasquez
Aquatic Ecotoxicologist



Pacific EcoRisk is accredited in accordance with NELAP (ORELAP ID 4043). Pacific EcoRisk certifies that the test results reported herein conform to the most current NELAP requirements for parameters for which accreditation is required and available. Any exceptions to NELAP requirements are noted, where applicable, in the body of the report. This report shall not be reproduced, except in full, without the written consent of Pacific EcoRisk. This testing was performed under Lab Order 22017.

**NPDES Compliance Toxicity Testing of the
Municipality of Anchorage John M. Asplund
Water Pollution Control Facility Effluent:
Annual Species Screening**

Samples collected February 17, 19, and 21, 2014

Performed For:

Kinnetic Laboratories, Inc.
1102 West 7th Avenue
Anchorage, AK 99501

Prepared By:

Pacific EcoRisk
2250 Cordelia Rd.
Fairfield, CA 94534

March 2014



PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

**NPDES Compliance Toxicity Testing of the Municipality of Anchorage
John M. Asplund Water Pollution Control Facility Effluent:
Annual Species Screening**

Samples collected February 17, 19, and 21, 2014

Table of Contents

	Page
1. INTRODUCTION	1
2. TOXICITY TEST PROCEDURES	1
2.1 Receipt and Handling of the Effluent Samples	1
2.2 Echinoderm Fertilization Toxicity Testing with <i>Strongylocentrotus purpuratus</i>	2
2.2.1 Reference Toxicant Testing of the Purple Urchins	2
2.3 Bivalve Embryo Development Toxicity Testing with <i>Mytilus galloprovincialis</i>	3
2.3.1 Reference Toxicant Testing of the <i>Mytilus galloprovincialis</i>	4
2.4 Survival and Growth Toxicity Testing with Topsmelt (<i>Atherinops affinis</i>)	4
2.4.1 Reference Toxicant Testing of the Topsmelt	5
3. RESULTS	6
3.1 Effects of Anchorage Effluent on Purple Urchins	6
3.1.1 Reference Toxicant Toxicity to the Purple Urchin	6
3.2 Effects of Anchorage Effluent on <i>Mytilus galloprovincialis</i>	7
3.2.1 Reference Toxicant Toxicity to <i>Mytilus galloprovincialis</i>	7
3.3 Effects of Anchorage Effluent on Topsmelt	8
3.3.1 Reference Toxicant Toxicity to Topsmelt	9
4. SUMMARY AND CONCLUSIONS	10
4.1 QA/QC Summary	10

Appendices

- Appendix A Chain-of-Custody Records for the Collection and Delivery of the Anchorage Effluent Samples
- Appendix B Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to Purple Urchin Sperm Fertilization
- Appendix C Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Purple Urchin Sperm
- Appendix D Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to *Mytilus galloprovincialis* Embryos
- Appendix E Test Data and Summary of Statistics for the Reference Toxicant Evaluation of *Mytilus galloprovincialis* Embryos
- Appendix F Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to Topsmelt (*Atherinops affinis*)
- Appendix G Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Topsmelt (*Atherinops affinis*)

1. INTRODUCTION

Kinnetic Laboratories, Inc., (Kinnetic) has contracted Pacific EcoRisk (PER) to perform evaluations of the chronic toxicity of effluent collected from the Municipality of Anchorage John M. Asplund Water Pollution Control Facility (Anchorage). The Anchorage NPDES permit states that "each year the permittee shall re-screen for one quarter with three species and continue to monitor for the rest of the year with the most sensitive species". Accordingly, the current testing comprises this annual species screening, and consisted of performing the following US EPA chronic toxicity tests:

- an echinoderm sperm fertilization test with the purple urchin, *Strongylocentrotus purpuratus*;
- an embryo-development test with the mussel, *Mytilus galloprovincialis*; and
- a 7-day survival and growth test with topsmelt, *Atherinops affinis*.

These tests were performed using effluent samples collected on February 17, 19, and 21, 2014. In order to assess the sensitivity of the test organisms to chronic toxic stress, reference toxicant tests were also performed. This report describes the performance and results of these effluent and reference toxicant tests.

2. TOXICITY TEST PROCEDURES

The methods used in conducting this toxicity testing followed the guidelines established by the EPA manuals " Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms " (EPA/600/R-95/136).

2.1 Receipt and Handling of the Effluent Samples

On February 17, 19 and 21, samples of Anchorage effluent were collected into appropriately cleaned sample containers; these samples were shipped via overnight delivery, on ice and under chain-of-custody, to the PER testing facility in Fairfield, CA. Upon receipt at the testing laboratory, aliquots of the samples were collected for determination of initial water quality characteristics (Table 1), after which the remainder of the sample was stored at 0-6°C, except when being used to prepare the test solutions. The chain-of-custody record for the collection and delivery of the samples are provided in Appendix A.

Table 1. Initial water quality characteristics of the Anchorage effluent sample.

Sample Collection Date	Sample Receipt Date	Sample ID	Temp (°C)	pH	D.O (mg/L)	Salinity (ppt)	Conductivity (µS/cm)	Total Ammonia (mg/L N)
2/17/14	2/18/14	MOA14TOX001	1.7	7.32	6.0	0.4	754	24.7
2/19/14	2/20/14	MOA14TOX002	1.4	7.25	6.8	0.4	773	25.8
2/21/14	2/22/14	MOA14TOX003	3.3	7.32	5.6	0.4	782	27.6

2.2 Echinoderm Fertilization Toxicity Testing with *Strongylocentrotus purpuratus*

The short-term chronic echinoderm sperm cell fertilization test consists of a bioassay in which purple sea urchin or sand dollar sperm are exposed to a series of effluent dilutions, and the subsequent effects on successful fertilization of the eggs determined. The specific procedures used in this test are described below.

The Lab Water Control medium for this test consisted of filtered (1 μm) seawater (collected from the UC Granite Canyon Marine Lab). The Lab Water Control medium and effluent sample were used to prepare test solutions at concentrations of 0.175, 0.35, 0.7, 1.4, and 2.8% effluent. Routine water quality characteristics (pH, D.O., and salinity) were measured for each test solution prior to use in this test.

Sperm and eggs were generated from gravid adult purple urchins, *S. purpuratus*. The gravid adult urchins were obtained from a commercial supplier (David Gutoff, San Diego, CA). Upon receipt at the PER lab, the urchins were held in tanks of aerated, filtered seawater at 12°C. Spawning of the urchins was induced by injection with 0.5 M KCl, followed by vigorous shaking of the animals to stimulate gamete release, as per EPA guidelines. The gametes from each spawning individual were collected and examined microscopically; the gametes exhibiting the best quality (as determined from morphology and trial fertilization) were pooled to provide a composite of high quality sperm and a composite of high quality eggs.

Each test replicate consisted of a 30-mL glass vial to which 5 mL of appropriate test solution was added. The test was initiated with the inoculation of an appropriate quantity of sperm into each replicate vial to achieve a final sperm-to-egg ratio of 500:1. After a 20-min exposure period, ~1000 eggs were inoculated into each vial. After an additional 20-min exposure, the test was terminated with all of the test embryos being fixed by the addition of 1.0 mL of 5% glutaraldehyde.

The contents of each preserved test vial were subsequently examined microscopically to determine the percentage of embryos exhibiting complete fertilization. The resulting percentage fertilization data for each test treatment were analyzed in order to characterize any statistically significant reductions in successful fertilization that may have been caused by the effluent; determinations of the key statistical endpoints were made using the CETIS[®] statistical software.

2.2.1 Reference Toxicant Testing of the Purple Urchins

In order to assess the sensitivity of the urchin sperm to toxicant stress, a reference toxicant test was performed concurrently with the effluent test. The reference toxicant test was performed similarly to the effluent test, but used test solutions consisting of Lab Water Control medium spiked with KCl at concentrations of 0.25, 0.5, 1, 2, and 4 g/L KCL. The resulting test response data were analyzed to determine key dose-response point estimates (e.g., EC₅₀); all statistical

analyses were made using the CETIS® software. These response endpoints were then compared to the “typical response” range established by the mean \pm 2 SD of the point estimates generated by the reference toxicant test database.

2.3 Bivalve Embryo Development Toxicity Testing with *Mytilus galloprovincialis*

The short-term chronic bivalve embryo development test consists of a ~48 hr bioassay in which mussel (*M. galloprovincialis*) embryos are exposed to a series of effluent dilutions, and the effects on embryo development determined. The specific procedures used in this test are described below.

The Lab Water Control medium for this test consisted of 1- μ m filtered seawater (collected from the UC Granite Canyon Marine Laboratory) diluted to a salinity of ~30 ppt with Type 1 lab water (reverse-osmosis, de-ionized water). The Lab Water Control medium and effluent sample were used to prepare a dilution series of treatment test solutions at test concentrations of 0.175, 0.35, 0.7, 1.4, and 2.8% effluent. Routine water quality characteristics (pH, D.O., and salinity) were measured for each test solution prior to use in this test.

Bivalve embryos were generated from gravid adult *M. galloprovincialis*. Prior to spawning, the adult bivalves were held in seawater at a temperature of 12°C. To induce spawning, the adults were placed into glass trays of clean seawater (filtered Granite Canyon seawater) at 20°C. This increase in temperature induced the bivalves to release sperm and eggs. When an individual was observed to begin releasing sperm or eggs, it was transferred to a separate container for isolation and collection of gametes, which were examined microscopically to evaluate viability and quality. The gametes exhibiting the best quality were used to prepare freshly fertilized embryos.

Each test replicate consisted of a 30-mL glass vial containing 10 mL of appropriate test solution. Additional replicates were also established to verify the inoculation density, and additional “observation” vials were established at the natural seawater Lab Control treatment for monitoring of successful embryo development (i.e., to allow monitoring of the test conditions without affecting actual test replicates). Finally, “water quality” vials (30-mL vials containing 20 mL of test solution at the same embryo density as the test vials) were established for each treatment in order to measure the final (~48 hrs) water quality characteristics.

The test was initiated with the random inoculation of approximately 150-300 embryos into each vial. These test, observation, and monitoring vials were then placed into a temperature-controlled incubator at 18°C under a 16L:8D photoperiod.

After 48 (\pm 1) hrs, the “observation” vials were examined to ensure that \geq 90% of the surviving embryos achieved normal development to the “D-hinge” stage. Upon confirming adequate successful embryo development, it was assumed that similar conditions existed for the test Lab

Control replicates, and the test was terminated. The final water quality characteristics were determined from the "water quality" vial at each treatment, and the remaining test embryos were fixed by the addition of 1 mL of 5% glutaraldehyde to each replicate vial. The contents of each preserved test vial were subsequently examined microscopically to determine the percentage of embryos exhibiting normal development. The resulting embryo development data were analyzed to evaluate any impairments due to the effluent; all statistical analyses were performed using the CETIS® statistical software.

2.3.1 Reference Toxicant Testing of the *Mytilus galloprovincialis*

In order to assess the sensitivity of the mussel embryos to toxic stress, a reference toxicant test was performed. This reference toxicant test was performed similarly to the effluent toxicity test, except that test solutions consisted of Lab Water Control medium (30 ppt seawater) spiked with KCl at concentrations of 0.5, 1, 2, 3, and 4 g/L. The resulting test response data were analyzed to determine key dose-response point estimates (e.g., EC₅₀); all statistical analyses were made using the CETIS® software. These response endpoints were then compared to the "typical response" range established by the mean \pm 2 SD of the point estimates generated by the reference toxicant test database

2.4 Survival and Growth Toxicity Testing with Topsmelt (*Atherinops affinis*)

The short-term chronic topsmelt test consists of exposing larval fish to a series of effluent dilutions for 7 days, after which effects on survival and growth are evaluated. The specific procedures used in this test are described below.

The larval topsmelt used in this test were obtained from a commercial supplier (Aquatic Biosystems, Fort Collins, CO). Upon receipt at the testing lab, the larval fish were maintained in a tank containing aerated Lab Water Control medium. The fish were fed brine shrimp nauplii *ad libitum* during the pre-test holding period.

The Lab Water Control medium for this test consisted of Type 1 water (reverse-osmosis, de-ionized water) diluted to a salinity of 25 ppt using an artificial sea salt (Crystal Sea Salt®-bioassay grade). Aliquots of the effluent were similarly adjusted to 25 ppt using the same sea salt. The Lab Water Control medium and effluent were used to prepare daily test solutions at concentrations of 0.175, 0.35, 0.7, 1.4, and 2.8% effluent. "New" water quality characteristics (pH, D.O., and salinity) were measured on these test solutions prior to use in the test.

There were 5 replicates each for the each treatment, each replicate consisting of 400 mL of test media in a 600-mL glass beaker. The test was initiated by randomly allocating five 12-day old topsmelt into each replicate beaker. The beakers were randomly positioned in a temperature-controlled room at 20°C (with temperature being monitored daily), under a 16L:8D photoperiod. These test fish were fed brine shrimp nauplii twice daily.

Each day of the test, fresh test solutions were prepared as before. The test replicate beakers were examined, with any dead animals, uneaten food, wastes, and other detritus being removed. The number of live fish in each replicate was determined and then approximately 80% of the test media in each beaker was carefully poured out and replaced with fresh media. "Old" water quality characteristics (pH, D.O., and salinity) were measured on the old test water collected from one randomly selected replicate at each treatment. The test beakers were then placed back into the temperature-controlled room.

After 7 days exposure, the test was terminated and the number of live fish in each replicate beaker was recorded. The fish from each replicate were then carefully euthanized in methanol, rinsed in de-ionized water, and transferred to a pre-dried and pre-tared weighing pan. The fish were then dried at 100°C for >24 hrs and re-weighed to determine the total weight of fish in each replicate; the total weight was then divided by the initial number of fish per replicate (n=5) to determine the "biomass value". The resulting survival and growth (biomass value) data were analyzed to determine any impairment(s) caused by the effluent; all statistical analyses were performed using the CETIS® statistical software.

2.4.1 Reference Toxicant Testing of the Topsmelt

In order to assess the sensitivity of the fish test organisms to toxic stress, a reference toxicant test was performed concurrently with the effluent test. The reference toxicant test was performed similarly to the effluent test, but uses test solutions consisting of Lab Water Control medium spiked with KCl at concentrations of 0.25, 0.5, 0.75, 1.0, 1.25 g/L. The resulting test response data were analyzed to determine key dose-response point estimates (e.g., EC₅₀); all statistical analyses were made using the CETIS® software. These response endpoints were then compared to the 'typical response' range established by the mean \pm 2 SD of the point estimates generated by the most recent previous reference toxicant tests performed by this lab.

3. RESULTS

3.1 Effects of Anchorage Effluent on Purple Urchins

The results of this test are summarized below in Table 2. The normal embryo fertilization NOEC was 1.4% effluent, resulting in 71.4 TUC (where TUC = 100/NOEC). The test data and summary of statistical analyses for this test are presented in Appendix B.

Table 2. Effects of Anchorage effluent on echinoderm (purple urchin) sperm fertilization.	
Effluent Treatment	Mean % Successful Fertilization
Lab Control (Filtered Seawater)	99.5
0.175%	100
0.35%	99.8
0.7%	99.8
1.4%	100
2.8%	87.8*
Summary of Key Statistics	
NOEC =	1.4% effluent
TUC (where TUC = 100/NOEC) =	71.4
EC ₁₅ =	>2.8% effluent
EC ₂₅ =	>2.8% effluent
EC ₄₀ =	>2.8% effluent
EC ₅₀ =	>2.8% effluent

* - The response at this test treatment was significantly less than the Lab Control treatment response at $p < 0.05$.

3.1.1 Reference Toxicant Toxicity to the Purple Urchin

The results of this test are summarized below in Table 3. The EC₅₀ for this test was consistent with the “typical response” range established by the reference toxicant database for this species, indicating that these organisms were responding to toxic stress in a typical and consistent fashion. The test data & summary of statistical analyses for this test are presented in Appendix C.

Table 3. Reference toxicant testing: Effects of KCl on purple urchin sperm fertilization.	
KCl Treatment (g/L)	Mean % Normal Fertilization
Lab Control	100
0.25	100
0.5	100
1	94.0*
2	52.5*
4	0*
Summary of Key Statistics	
EC ₅₀ =	1.5 g/L KCl

* - The response at this test treatment was significantly less than the Lab Control treatment response at $p < 0.05$.

3.2 Effects of Anchorage Effluent on *Mytilus galloprovincialis*

The results for this test are summarized below in Table 4. The normal embryo development NOEC was 2.8% effluent, resulting in 35.7 TUC (where TUC = 100/NOEC). The test data and summary of statistical analyses for this test are attached as Appendix D.

Table 4. Effects of Anchorage effluent on <i>Mytilus galloprovincialis</i> embryo development.	
Effluent Treatment	Mean % Normal Embryo Development
Lab Water Control (Seawater @ 30 ppt)	99.6
0.175%	99.4
0.35%	98.6
0.7%	98.9
1.4%	99.1
2.8%	98.8
Summary of Key Statistics	
NOEC =	2.8% effluent
TUC (100/NOEC) =	35.7
EC15 =	>2.8% effluent ^a
EC25 =	>2.8% effluent ^a
EC40 =	>2.8% effluent ^a
EC50 =	>2.8% effluent ^a

a - Due to the absence of significant impairment, the EC point estimates could not be calculated, but can be determined by inspection to be >2.8% effluent.

3.2.1 Reference Toxicant Toxicity to *Mytilus galloprovincialis*

The results of this test are summarized below in Table 5. The EC50 for this test was consistent with the "typical response" range established by the reference toxicant test database for this species, indicating that the organisms used in this testing were responding to toxic stress in a typical fashion. The test data and summary of statistics for this test are attached as Appendix E.

Table 5. Reference toxicant testing: Effects of KCl on mussel embryo development.	
KCl Treatment (g/L)	Mean % Normal Embryo Development
Lab Water Control	98.9
0.5	99.7
1	99.9
2	98.8
3	0*
4	0*
Summary of Key Statistics	
EC50 =	2.4 g/L KCl

* - The response at this test treatment was significantly less than the Lab Control treatment response ($p < 0.05$).

3.3 Effects of Anchorage Effluent on Topsmelt

The results of this test are presented in Table 6. There were no significant reductions in survival or growth at the Anchorage effluent concentrations tested; the NOEC was 2.8% effluent for both test endpoints, resulting in 35.7 TUC (where TUC = 100/NOEC). The test data and the summary of statistical analyses for this test are attached as Appendix F.

Table 6. Effects of Anchorage effluent on topsmelt survival and growth.		
Effluent Treatment	Mean % Survival	Mean Biomass Value (mg)
Lab Water Control	100	1.56
0.175%	100	1.14 ^b
0.35%	100	1.39
0.7%	100	1.28
1.4%	100	1.28
2.8%	100	1.26
Summary of Key Statistics		
NOEC =	2.8% effluent	2.8% effluent
TUC (100/NOEC) =	35.7	35.7
Survival EC15 or Growth IC15 =	>2.8% effluent ^a	>2.8% effluent
Survival EC25 or Growth IC25 =	>2.8% effluent ^a	>2.8% effluent
Survival EC40 or Growth IC40 =	>2.8% effluent ^a	>2.8% effluent
Survival EC50 or Growth IC50 =	>2.8% effluent ^a	>2.8% effluent

a - Due to the absence of significant mortalities, the EC point estimates could not be calculated, but can be determined by inspection to be >2.8% effluent.

b - There was an interrupted concentration-response, with an indication of a statistically significant reduction in test response at this test treatment; however, the absence of statistically significant reductions at the higher effluent concentrations indicates that the reduction observed at this treatment is not toxicologically significant.

3.3.1 Reference Toxicant Toxicity to Topsmelt

The results of this test are summarized below in Table 7. The EC₅₀ and IC₅₀ for this test were consistent with the “typical response” ranges established by the reference toxicant test database for this species, indicating that the organisms used in these tests were responding to toxic stress in a typical fashion. The test data and summary of statistics for this test are attached as Appendix G.

Table 7. Reference toxicant testing: Effects of KCl on topsmelt.		
KCl Concentration (g/L)	Mean % Survival	Mean Biomass Value (mg)
Lab Water Control	100	1.39
0.25	100	1.38
0.5	100	1.33
0.75	100	1.25
1	24*	0.24
1.25	0*	-
Summary of Key Statistics		
Survival EC ₅₀ or Growth IC ₅₀ =	0.92 g/L KCl	0.89 g/L KCl

* - The response at this test treatment was significantly less than the Lab Control treatment response ($p < 0.05$).

4. SUMMARY AND CONCLUSIONS

Chronic Effects of Anchorage Effluent on Purple Urchin Sperm Fertilization

There were significant reductions in successful fertilization at the 2.8% effluent concentration tested; the NOEC was 1.4% effluent, resulting in 71.4 TUc.

Chronic Effects of Anchorage Effluent on *Mytilus galloprovincialis* Embryo Development

There were no significant reductions in normal embryo development at the any of effluent concentrations tested; the NOEC was 2.8% effluent, resulting in 35.7 TUc.

Chronic Effects of Anchorage Effluent on Topsmelt (*Atherinops affinis*)

There were no significant reductions in survival or growth at the any of effluent concentrations tested; the NOEC was 2.8% effluent for both test endpoints, resulting in 35.7 TUc.

4.1 QA/QC Summary

Test Conditions – All test conditions (pH, D.O., temperature, etc.) were within acceptable limits. All analyses were performed according to laboratory Standard Operating Procedures.

Negative Control – The test organism responses at the Lab Control treatments were within acceptable limits.

Positive Control – The reference toxicant test results for all three species were within the “typical response” ranges established by the respective reference toxicant test databases for these species, indicating that these test organisms were responding to toxicant stress in a typical and consistent fashion.

Concentration Response Relationships – The concentration-response relationships for these tests were evaluated as per EPA guidelines (EPA-821-B-00-004), and were determined to be acceptable.

Appendix A



Chain-of-Custody Records for the Collection and Delivery of the Anchorage Effluent Samples

KINETIC LABORATORIES, INC. CHAIN OF CUSTODY RECORD

LABORATORY:		FROM:			
ATTN: Scott Ogle 707-207-7760 Pacific EcoRisk 2250 Cordelia Rd. Fairfield, CA 94534		Kinnetic Laboratories, Inc. 704 W. 2 nd Ave Anchorage, AK 99501 Attn: Gary Lawley 907.276.6178			
Required Completion Date: ASAP		P.O. #: AK14-1000	KLI Proj. #: 516.03		
ANALYSIS TYPE: Static renewal chronic testing using 1) <i>Atherinops affinas</i> (Topsmelt), survival and growth test, 2) <i>Mytilus spp.</i> , survival and growth test, and 3) <i>Stronglocentruss purpuratus</i> (Purple urchin) fertilization test. Test following procedures in accordance with <i>Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms</i> , (EPA/600/4-87/028). At least five dilutions must be tested, including a ZID boundary concentration of 0.70%, two concentrations below 0.70% (0.35 and 0.175) and two concentrations above (1.4 and 2.8%).					
		Preservative: None (4° C)	Type of Container: 1 gallon cubitainer		
SAMPLE IDENTIFICATION #	NO. OF CONTAINERS	SAMPLE DATE	SAMPLE TIME	CONDITION UPON RECEIPT	ASSIGNED LABORATORY NUMBER
MOA14TOX001	1	2/17/14	0600		
DATA REPORTS MUST INCLUDE THE FOLLOWING: SAMPLE ID NUMBER, ANALYTICAL METHOD, DETECTION LIMIT, DATE OF EXTRACTION, DATE OF ANALYSIS, ANALYTICAL RESULTS, AND SIGNATURE OF QA REVIEWER.					
COMMENTS: Please return all completed original COCs to KLI Anchorage.					
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME	
[Signature]	2/17/14 1300	Fruit Ex	[Signature]	2/18/14 1115	
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME	
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME	

SAMPLED BY (NAME/SIGNATURE):

KINETIC LABORATORIES, INC. CHAIN OF CUSTODY RECORD

LABORATORY:		FROM:			
ATTN: Scott Ogle 707-207-7760 Pacific EcoRisk 2250 Cordella Rd. Fairfield, CA 94534		Kinnetic Laboratories, Inc. 1102 West 7th Avenue Anchorage, AK 99501 Attn: Gary Lawley 907.276.6178			
Required Completion Date: ASAP		P.O. #: AK14-1000	KLI Proj. #: 516.03		
ANALYSIS TYPE: Static renewal chronic testing using 1) <i>Atherinops affinas</i> (Topsmelt), survival and growth test, 2) <i>Mytilus spp.</i> , survival and growth test, and 3) <i>Stronglocentruss purpuratus</i> (Purple urchin). fertilization test. Test following procedures in accordance with <i>Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms</i> , (EPA/600/4-87/028). At least five dilutions must be tested, including a ZID boundary concentration of 0.70%, two concentrations below 0.70% (0.35 and 0.175) and two concentrations above (1.4 and 2.8%).					
		Preservative: None (4°C)	Type of Container: 2.5 gallon cubitainer		
SAMPLE IDENTIFICATION #	NO. OF CONTAINERS	SAMPLE DATE	SAMPLE TIME	CONDITION UPON RECEIPT	ASSIGNED LABORATORY NUMBER
MOA14TOX002	1	2/19/14	0900		
DATA REPORTS MUST INCLUDE THE FOLLOWING: SAMPLE ID NUMBER, ANALYTICAL METHOD, DETECTION LIMIT, DATE OF EXTRACTION, DATE OF ANALYSIS, ANALYTICAL RESULTS, AND SIGNATURE OF QA REVIEWER.					
COMMENTS: Please return all completed original COCs to KLI Anchorage.					
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME	
	2/19/14 1500	Fel Ex	 TM	2/20/14 104	
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME	
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME	

SAMPLED BY (NAME/SIGNATURE):

Appendix B

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to Purple Urchin Sperm Fertilization

CETIS Summary Report

Report Date: 18 Feb-14 17:45 (p 1 of 1)
Test Code: 55115 | 16-6525-4287

Echinoid Fertilization Test						Pacific EcoRisk					
Batch ID:	18-0878-1680	Test Type:	Fertilization	Analyst:	Stevi Vasquez						
Start Date:	18 Feb-14 15:46	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Filtered Sea Water						
Ending Date:	18 Feb-14 16:26	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Gutloff	Age:	NA						
Sample ID:	21-4061-0777	Code:	Effluent	Client:	Kinnetic Labs						
Sample Date:	17 Feb-14 06:00	Material:	Effluent	Project:	22017						
Receive Date:	18 Feb-14 11:15	Source:	Kinnetic Laboratories, Inc.								
Sample Age:	34h (1.7 °C)	Station:	MOA14TOX001								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
12-0486-3237	Fertilization Rate	1.4	2.8	1.98	3.28%	71.43	Steel Many-One Rank Sum Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
01-1296-0437	Fertilization Rate	EC5	1.98	1.59	N/A	50.51	Linear Interpolation (ICPIN)				
		EC10	2.58	1.78	N/A	39.07					
		EC15	>2.8	N/A	N/A	<35.71					
		EC20	>2.8	N/A	N/A	<35.71					
		EC25	>2.8	N/A	N/A	<35.71					
		EC40	>2.8	N/A	N/A	<35.71					
		EC50	>2.8	N/A	N/A	<35.71					
Fertilization Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.995	0.993	0.997	0.99	1	0.00289	0.00577	0.58%	0.0%
0.175		4	1	1	1	1	1	0	0	0.0%	-0.5%
0.35		4	0.998	0.996	0.999	0.99	1	0.0025	0.005	0.5%	-0.25%
0.7		4	0.998	0.996	0.999	0.99	1	0.0025	0.005	0.5%	-0.25%
1.4		4	1	1	1	1	1	0	0	0.0%	-0.5%
2.8		4	0.878	0.84	0.915	0.79	0.97	0.0506	0.101	11.5%	11.8%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	1	0.99	1	0.99						
0.175		1	1	1	1						
0.35		0.99	1	1	1						
0.7		1	1	0.99	1						
1.4		1	1	1	1						
2.8		0.96	0.79	0.79	0.97						
Fertilization Rate Binomials											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	100/100	99/100	100/100	99/100						
0.175		100/100	100/100	100/100	100/100						
0.35		99/100	100/100	100/100	100/100						
0.7		100/100	100/100	99/100	100/100						
1.4		100/100	100/100	100/100	100/100						
2.8		96/100	79/100	79/100	97/100						

CETIS Analytical Report

Report Date: 18 Feb-14 17:45 (p 1 of 2)

Test Code: 55116 | 16-6525-4287

Echinoid Fertilization Test Pacific EcoRisk

Analysis ID: 12-0486-3237 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.5
 Analyzed: 18 Feb-14 17:45 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.28%	1.4	2.8	1.98	71.43

Steel Many-One Rank Sum Test									
Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Water Control		0.175	22	10	2	6	0.9908	Asymp	Non-Significant Effect
		0.35	20	10	3	6	0.9516	Asymp	Non-Significant Effect
		0.7	20	10	3	6	0.9516	Asymp	Non-Significant Effect
		1.4	22	10	2	6	0.9908	Asymp	Non-Significant Effect
		2.8*	10	10	0	6	0.0417	Asymp	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2480599	0.04961199	5	9.95	0.0001	Significant Effect
Error	0.08978323	0.004987957	18			
Total	0.3378431		23			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	51.3	4.25	<0.0001	Unequal Variances
Variances	Levene Equality of Variance	164	4.25	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.797	0.884	0.0003	Non-normal Distribution

Fertilization Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Contr	4	0.995	0.986	1	0.995	0.99	1	0.00289	0.58%	0.0%
0.175		4	1	1	1	1	1	1	0	0.0%	-0.5%
0.35		4	0.998	0.99	1	1	0.99	1	0.0025	0.5%	-0.25%
0.7		4	0.998	0.99	1	1	0.99	1	0.0025	0.5%	-0.25%
1.4		4	1	1	1	1	1	1	0	0.0%	-0.5%
2.8		4	0.878	0.717	1	0.875	0.79	0.97	0.0506	11.5%	11.8%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Cont	4	1.5	1.45	1.54	1.5	1.47	1.52	0.0145	1.94%	0.0%
0.175		4	1.52	1.52	1.52	1.52	1.52	1.52	0	0.0%	-1.68%
0.35		4	1.51	1.47	1.55	1.52	1.47	1.52	0.0125	1.66%	-0.84%
0.7		4	1.51	1.47	1.55	1.52	1.47	1.52	0.0125	1.66%	-0.84%
1.4		4	1.52	1.52	1.52	1.52	1.52	1.52	0	0.0%	-1.68%
2.8		4	1.24	0.973	1.5	1.23	1.09	1.4	0.0834	13.5%	17.2%

CETIS Analytical Report

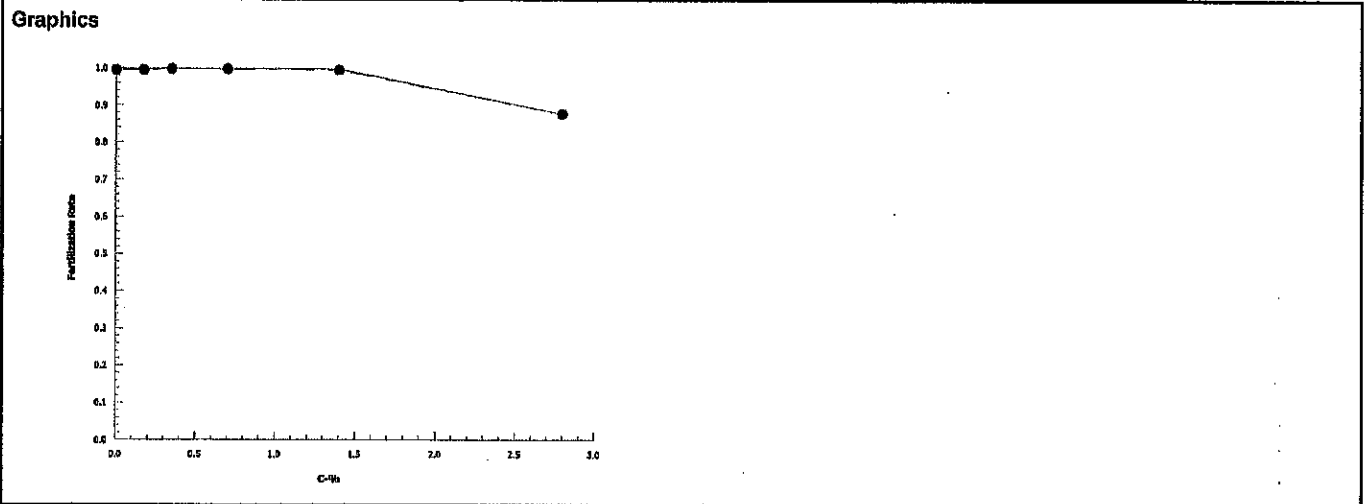
Report Date: 18 Feb-14 17:45 (p 1 of 1)
Test Code: 55115 | 16-6525-4287

Echinoid Fertilization Test				Pacific EcoRisk	
Analysis ID:	01-1296-0437	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.5
Analyzed:	18 Feb-14 17:45	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2026224	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC5	1.98	1.59	N/A	50.51	NA	62.91
EC10	2.56	1.78	N/A	39.07	NA	56.21
EC15	>2.8	N/A	N/A	<35.71	NA	NA
EC20	>2.8	N/A	N/A	<35.71	NA	NA
EC25	>2.8	N/A	N/A	<35.71	NA	NA
EC40	>2.8	N/A	N/A	<35.71	NA	NA
EC50	>2.8	N/A	N/A	<35.71	NA	NA

Fertilization Rate Summary			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Water Contr	4	0.995	0.99	1	0.00289	0.00577	0.58%	0.0%	398	400
0.175		4	1	1	1	0	0	0.0%	-0.5%	400	400
0.35		4	0.998	0.99	1	0.0025	0.005	0.5%	-0.25%	399	400
0.7		4	0.998	0.99	1	0.0025	0.005	0.5%	-0.25%	399	400
1.4		4	1	1	1	0	0	0.0%	-0.5%	400	400
2.8		4	0.878	0.79	0.97	0.0506	0.101	11.5%	11.8%	351	400



Echinoderm Fertilization Toxicity Test Data Sheet

Client: Kinnetic Anchorage
 Test Material: Effluent
 Test Species: *S. purpuratus*
 Test ID #: 55115
 Project #: 22017

Test Start Date: 2/18/14
 Test End Date: 2/18/14
 Enumeration Date: 2/18/14
 Investigator: SVV

Sample Salinity adjusted with : —

Concentration	Replicate	Number of Fertilized Eggs	Number of Unfertilized Eggs	Total Number of Eggs	Percent Fertilization
Control	A	100	0	100	100
	B	99	1	100	99
	C	100	0	100	100
	D	99	1	100	99
0.175%	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	100	0	100	100
0.35%	A	99	1	100	99
	B	100	0	100	100
	C	100	0	100	100
	D	100	0	100	100
0.7%	A	100	0	100	100
	B	100	0	100	100
	C	99	1	100	99
	D	100	0	100	100
1.4%	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	100	0	100	100
2.8%	A	96	4	100	96
	B	79	21	100	79
	C	79	21	100	79
	D	97	3	100	97

Echinoderm Fertilization Toxicity Test Water Chemistry Data

Client: Kinnetic Anchorage
 Test Material: Effluent
 Test Species: *S. purpuratus*
 Test ID#: 55115 Project #: 22017
 Sample Salinity adjusted with: ---

Organism Log#: 7968 Age: N/A
 Organism Supplier: M-Rep
 Control/Diluent: FSW
 Test Date: 2/18/14 Randomization: ---

Treatment	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	11.3	7.77	8.8	33.6	Date: 2/18/14
0.175%	11.3	7.79	8.8	33.6	Sample ID: 34100
0.35%	11.3	7.79	8.9	33.6	Test Solution Prep: 8V
0.7%	11.3	7.79	8.8	33.7	New WQ: CJD
1.4%	11.3	7.78	8.8	33.4	Inoculation Time: 1546
2.8%	11.3	7.76	8.7	33.0	Inoculation Signoff: 8V
Meter ID	78A	PH15	RD04	EC04	

Appendix C

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Purple Urchin Sperm

CETIS Summary Report

 Report Date: 18 Feb-14 17:50 (p 1 of 1)
 Test Code: 55118 | 13-4044-9511

Echinoid Fertilization Test							Pacific EcoRisk				
Batch ID:	10-7994-3253	Test Type:	Fertilization	Analyst:	Stevi Vasquez						
Start Date:	18 Feb-14 15:47	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Filtered Sea Water						
Ending Date:	18 Feb-14 16:27	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Dave Gutoff	Age:	NA						
Sample ID:	05-9833-4304	Code:	KCl	Client:	Reference Toxicant						
Sample Date:	18 Feb-14 15:47	Material:	Potassium chloride	Project:	22018						
Receive Date:	18 Feb-14 15:47	Source:	Reference Toxicant								
Sample Age:	NA (11.3 °C)	Station:	In House								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
20-7403-1605	Fertilization Rate	0.5	1	0.7071	2.68%		Steel Many-One Rank Sum Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	g/L	95% LCL	95% UCL	TU	Method				
15-4618-0369	Fertilization Rate	EC5	0.917	0.64	1.1		Linear Interpolation (ICPIN)				
		EC10	1.05	0.953	1.11						
		EC15	1.1	1.03	1.17						
		EC20	1.18	1.09	1.22						
		EC25	1.21	1.14	1.27						
		EC40	1.38	1.32	1.44						
		EC50	1.5	1.44	1.55						
Fertilization Rate Summary											
C-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	1	1	1	1	1	0	0	0.0%	0.0%
0.25		4	1	1	1	1	1	0	0	0.0%	0.0%
0.5		4	1	1	1	1	1	0	0	0.0%	0.0%
1		4	0.94	0.92	0.96	0.87	0.98	0.0261	0.0523	5.56%	6.0%
2		4	0.0525	0.0354	0.0696	0	0.1	0.0229	0.0457	87.1%	94.7%
4		4	0	0	0	0	0	0	0		100.0%
Fertilization Rate Detail											
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	1	1	1	1						
0.25		1	1	1	1						
0.5		1	1	1	1						
1		0.93	0.87	0.98	0.98						
2		0	0.08	0.03	0.1						
4		0	0	0	0						
Fertilization Rate Binomials											
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	100/100	100/100	100/100	100/100						
0.25		100/100	100/100	100/100	100/100						
0.5		100/100	100/100	100/100	100/100						
1		93/100	87/100	98/100	98/100						
2		0/100	8/100	3/100	10/100						
4		0/100	0/100	0/100	0/100						

CETIS QC Plot

Report Date: 18 Feb-14 17:51 (1 of 1)

Echinoid Fertilization Test

Pacific EcoRisk

Test Type: Fertilization

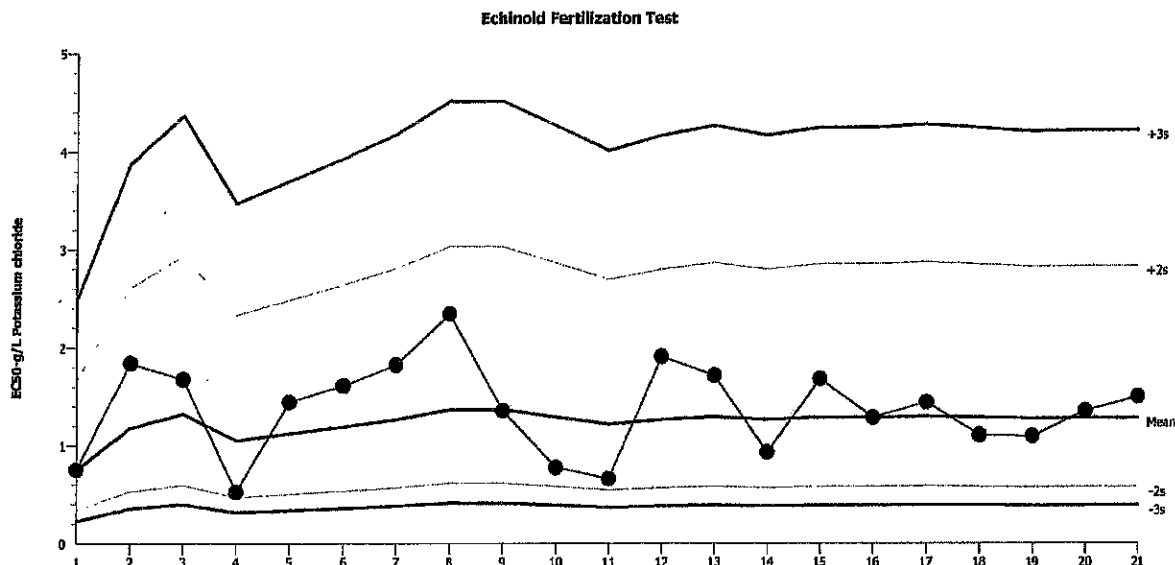
Organism: Strongylocentrotus purpuratus (Purpl

Material: Potassium chloride

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF



Mean: 1.275

Count: 20

-2s Warning Limit: 0.5755

-3s Action Limit: 0.3867

Sigma: NA

CV: 48.80%

+2s Warning Limit: 2.823

+3s Action Limit: 4.202

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2010	Apr	27	15:16	0.7522	-0.5225	-1.327			08-6375-6676	16-8011-5000
2	2011	Jul	5	16:59	1.841	0.5664	0.9247			12-7539-1611	11-4712-0703
3			5	16:59	1.678	0.4034	0.6915			08-6048-1952	03-9460-7936
4		Oct	14	15:51	0.5287	-0.746	-2.213	(-)		00-9189-5053	02-0698-6488
5		Dec	15	13:55	1.444	0.1698	0.3144			04-6051-4150	20-8134-1190
6	2012	Mar	8	16:22	1.61	0.3356	0.5878			05-0740-0748	15-5935-0686
7		Apr	6	15:30	1.823	0.5488	0.9005			04-2265-9762	19-9125-4309
8			25	18:20	2.342	1.068	1.53			10-8393-5625	06-2730-7786
9		Aug	10	15:00	1.354	0.07913	0.1515			01-9226-6824	09-0663-0632
10		Oct	5	17:17	0.7751	-0.4996	-1.251			13-9975-3204	21-3214-1203
11			25	17:20	0.661	-0.6136	-1.651			15-6047-1276	05-8563-4140
12	2013	Jan	31	16:20	1.905	0.6299	1.01			20-4482-2275	16-6771-7267
13		Apr	5	15:40	1.712	0.4371	0.7415			09-0614-8270	15-9800-9383
14		May	2	19:20	0.9268	-0.3479	-0.8016			10-7105-5755	13-0792-4685
15			7	15:58	1.677	0.4024	0.69			00-1706-4139	06-3677-7130
16		Aug	28	17:02	1.284	0.009512	0.0187			06-2696-3137	00-3784-6188
17		Nov	6	14:36	1.438	0.1634	0.3034			14-3825-5642	11-2685-4555
18		Dec	5	15:50	1.101	-0.1738	-0.3687			06-4350-3308	19-1664-9754
19	2014	Feb	7	14:20	1.09	-0.1845	-0.3933			11-6688-0585	16-9103-0015
20			8	18:00	1.348	0.07317	0.1404			02-9297-3328	08-5519-8625
21			18	15:47	1.496	0.2211	0.4023			13-4044-9511	15-4518-0369

Echinoderm Fertilization Reference Toxicant Test Data Sheet

Client: _____
 Test Material: _____
 Test Species: D. excentricus (S. purpuratus) (circle)
 Test ID #: 55118
 Project #: 22018

Test Start Date: 2/18/14
 Test End Date: 2/18/14
 Enumeration Date: 2/18/14
 Investigator: 8VV

Concentration (g/L KCl)	Replicate	Number of Fertilized Eggs	Number of Unfertilized Eggs	Total Number of Eggs	Percent Normal Fertilization
Control	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	100	0	100	100
0.25	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	100	0	100	100
0.5	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	100	0	100	100
1	A	93	7	100	93
	B	87	13	100	87
	C	98	2	100	98
	D	98	2	100	98
2	A	0	100	100	0
	B	8	92	100	8
	C	3	97	100	3
	D	10	90	100	10
4	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	0	100	100	0

Echinoderm Fertilization Reference Toxicant Test Water Chemistry Data

Client: Reference Toxicant
 Test Material: Potassium Chloride
 Test Species: *D. excentricus* (*S. purpuratus* (circle))
 Test ID#: 55118 Project #: 22018

Organism Log#: 7908 Age: N/A
 Organism Supplier: M-Rep
 Control/Diluent: FSW
 Test Date: 2/18/14 Randomization: -

Treatment (g/L KCl)	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	11.3	7.72	8.9	33.7	Date: <u>2/18/14</u>
0.25	11.3	7.77	8.6	34.1	Test Solution Prep: <u>SVV</u>
0.5	11.3	7.79	9.0	34.3	New WQ: <u>CJD</u>
1	11.3	7.79	8.8	34.8	Innoculation Time: <u>1547</u>
2	11.3	7.79	9.0	35.8	Innoculation Signoff: <u>SVV</u>
4	11.3	7.78	9.1	38.0	
Meter ID	78A	PH15	RD04	EC04	

Appendix D

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to *Mytilus galloprovincialis* Embryos

CETIS Summary Report

Report Date: 26 Feb-14 17:04 (p 1 of 1)
 Test Code: 55116 | 19-5226-5569

Bivalve Larval Survival and Development Test Pacific EcoRisk

Batch ID: 17-1143-7703	Test Type: Development-Survival	Analyst: Michelle Fong
Start Date: 20 Feb-14 14:45	Protocol: EPA/600/R-95/136 (1995)	Diluent: Diluted Seawater
Ending Date: 22 Feb-14 15:00	Species: Mytilus galloprovincialis	Brine: Not Applicable
Duration: 48h	Source: Gutoff	Age: N/A

Sample ID: 14-9304-4418	Code: Effluent	Client: Kinnetic Laboratories, Inc
Sample Date: 19 Feb-14 09:00	Material: Effluent	Project: 22017
Receive Date: 20 Feb-14 10:45	Source: Kinnetic Laboratories, Inc.	
Sample Age: 30h (1.4 °C)	Station: MOA14TOX002	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-2766-3265	Development Rate	2.8	>2.8	NA	1.23%	35.71	Dunnett Multiple Comparison Test

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.996	0.994	0.998	0.988	1	0.00278	0.00557	0.56%	0.0%
0.175		4	0.994	0.991	0.997	0.983	1	0.00407	0.00813	0.82%	0.14%
0.35		4	0.986	0.984	0.988	0.983	0.995	0.00287	0.00574	0.58%	0.96%
0.7		4	0.989	0.986	0.992	0.981	1	0.0041	0.00821	0.83%	0.71%
1.4		4	0.991	0.989	0.993	0.984	0.995	0.00262	0.00524	0.53%	0.51%
2.8		4	0.988	0.985	0.991	0.981	1	0.00436	0.00872	0.88%	0.8%

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	0.995	1	1	0.988
0.175		0.995	0.983	1	1
0.35		0.983	0.995	0.984	0.984
0.7		0.981	1	0.989	0.985
1.4		0.984	0.989	0.995	0.995
2.8		0.981	1	0.982	0.988

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	202/203	210/210	175/175	168/170
0.175		195/196	171/174	153/153	165/165
0.35		170/173	193/194	181/184	183/186
0.7		206/210	200/200	180/182	196/199
1.4		184/187	185/187	197/198	192/193
2.8		205/209	179/179	166/169	168/170

CETIS Analytical Report

Report Date: 26 Feb-14 17:04 (p 1 of 2)
 Test Code: 55116 | 19-5226-5569

Bivalve Larval Survival and Development Test										Pacific EcoRisk	
Analysis ID: 05-2766-3265		Endpoint: Development Rate		CETIS Version: CETISv1.8.5							
Analyzed: 26 Feb-14 17:03		Analysis: Parametric-Control vs Treatments		Official Results: Yes							
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	1.23%	2.8	>2.8	NA	35.71		
Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Water Control		0.175	0.288	2.41	0.066	6	0.7350	CDF	Non-Significant Effect		
		0.35	1.91	2.41	0.066	6	0.1210	CDF	Non-Significant Effect		
		0.7	1.38	2.41	0.066	6	0.2670	CDF	Non-Significant Effect		
		1.4	1.13	2.41	0.066	6	0.3676	CDF	Non-Significant Effect		
		2.8	1.54	2.41	0.066	6	0.2164	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.008233612		0.001646722		5	1.11	0.3886	Non-Significant Effect			
Error	0.02665167		0.001480649		18						
Total	0.03488529				23						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		1.45	15.1	0.9190	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.966	0.884	0.5716	Normal Distribution					
Development Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Contr	4	0.996	0.987	1	0.998	0.988	1	0.00278	0.56%	0.0%
0.175		4	0.994	0.981	1	0.997	0.983	1	0.00407	0.82%	0.14%
0.35		4	0.986	0.977	0.995	0.984	0.983	0.995	0.00287	0.58%	0.96%
0.7		4	0.989	0.976	1	0.987	0.981	1	0.0041	0.83%	0.71%
1.4		4	0.991	0.982	0.999	0.992	0.984	0.995	0.00262	0.53%	0.51%
2.8		4	0.988	0.974	1	0.985	0.981	1	0.00436	0.88%	0.8%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Cont	4	1.51	1.45	1.56	1.52	1.46	1.54	0.0173	2.29%	0.0%
0.175		4	1.5	1.43	1.57	1.51	1.44	1.53	0.0217	2.89%	0.52%
0.35		4	1.46	1.41	1.5	1.44	1.44	1.5	0.0144	1.97%	3.45%
0.7		4	1.47	1.4	1.54	1.46	1.43	1.54	0.0228	3.1%	2.5%
1.4		4	1.48	1.43	1.52	1.48	1.44	1.5	0.0135	1.83%	2.03%
2.8		4	1.47	1.39	1.54	1.45	1.43	1.53	0.0234	3.19%	2.77%

Mytilus sp. Development Toxicity Test Count Data

Client: Kinnetic Anchorage
 Test Material: Effluent
 Test ID #: 55116
 Project #: 22017

Test Start Date: 2/20/14
 Test End Date: 2/22/14
 Enumeration Date: 2/23/14
 Investigator: CA

Sample Salinity adjusted with : -

Effluent Concentration	Replicate	Number of Normal Larvae	Number of Abnormal Larvae	Total Number Larvae	Percent Normal Development
Control	A	202	1	203	99.5
	B	210	0	210	100.0
	C	175	0	175	100.0
	D	168	2	170	98.8
0.175%	A	195	1	196	99.5
	B	171	3	174	98.3
	C	153	0	153	100.0
	D	165	0	165	100.0
0.35%	A	170	3	173	98.3
	B	193	1	194	99.5
	C	181	3	184	98.4
	D	183	3	186	98.4
0.7%	A	206	4	210	98.1
	B	200	0	200	100.0
	C	180	2	182	98.9
	D	196	3	199	98.5
1.4%	A	184	3	187	98.4
	B	185	2	187	98.9
	C	197	1	198	99.5
	D	192	1	193	99.5
2.8%	A	205	4	209	98.1
	B	179	0	179	100.0
	C	166	3	169	98.2
	D	168	2	170	98.8

Mytilus sp. Development Toxicity Test Water Chemistry Data

Client: **Kinnetic- Anchorage**
 Test Material: **Effluent**
 Test ID#: **55116** Project #: **22017**
 Test Date: **2/20/14** Randomization: **—**
 Sample Salinity adjusted with: **—**

Organism Log#: **7960** Age: **N/A**
 Organism Supplier: **Gutroff**
 Control/Diluent: **Filtered Seawater @ 30 ppt**

Day 0					
Treatment (%)	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	18.5	7.74	8.4	29.4	Sample ID: 34143
0.175	18.5	7.77	8.6	29.5	Test Solution Prep: SS
0.35	18.5	7.78	8.5	29.4	New WQ: SS
0.7	18.5	7.79	8.6	29.3	Innoculation Date: 2/20/14
1.4	18.5	7.78	8.5	29.1	Innoculation Time: 1445
2.8	18.5	7.77	8.6	28.4	Innoculation Signoff: SS
Meter ID	69A	PH 19	RDO7	EC04	

Day 1					
Treatment (%)	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	18.3				Date: 2/21/14
0.175	18.3				Old WQ: MK
0.35	18.3				
0.7	18.3				
1.4	18.3				
2.8	18.3				
Meter ID	69A				

Day 2					
Treatment (%)	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	18.2	7.83	7.6	29.5	Termination Date: 2/22/14
0.175	18.2	7.81	7.6	29.2	Termination Time: 1500
0.35	18.2	7.84	7.7	29.6	Termination Signoff: MK
0.7	18.2	7.84	7.7	29.5	Old WQ: N.M.S.
1.4	18.2	7.84	7.7	29.4	
2.8	18.2	7.83	7.6	29.2	
Meter ID	69A	PH 19	RDO7	EC04	

Appendix E

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of *Mytilus galloprovincialis* Embryos

CETIS Summary Report

Report Date: 05 Mar-14 17:19 (p 1 of 1)
Test Code: 55411 | 06-5660-7780

Bivalve Larval Survival and Development Test							Pacific EcoRisk					
Batch ID:	11-3691-3057	Test Type:	Development-Survival				Analyst:	Padrick Anderson				
Start Date:	20 Feb-14 14:50	Protocol:	EPA/600/R-95/136 (1995)				Diluent:	Diluted Seawater				
Ending Date:	22 Feb-14 15:00	Species:	Mytilus galloprovincialis				Brine:	Not Applicable				
Duration:	48h	Source:	Gutloff				Age:	n/a				
Sample ID:	18-9753-2643	Code:	KCI				Client:	Reference Toxicant				
Sample Date:	20 Feb-14 14:50	Material:	Potassium chloride				Project:	22105				
Receive Date:	20 Feb-14 14:50	Source:	Reference Toxicant									
Sample Age:	NA (18.5 °C)	Station:	In House									
Comparison Summary												
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method					
14-8874-0236	Development Rate	2	3	2.449	1.13%		Dunnett Multiple Comparison Test					
Point Estimate Summary												
Analysis ID	Endpoint	Level	g/L	95% LCL	95% UCL	TU	Method					
08-8983-3999	Development Rate	EC50	2.44	2.43	2.45		Spearman-Kärber					
Development Rate Summary												
C-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect	
0	Lab Water Contr	4	0.989	0.986	0.992	0.978	0.995	0.00391	0.00781	0.79%	0.0%	
0.5		4	0.997	0.996	0.998	0.994	1	0.00154	0.00308	0.31%	-0.88%	
1		4	0.999	0.998	1	0.995	1	0.00134	0.00267	0.27%	-1.01%	
2		4	0.988	0.984	0.991	0.974	0.994	0.00471	0.00941	0.95%	0.07%	
3		4	0	0	0	0	0	0	0		100.0%	
4		4	0	0	0	0	0	0	0		100.0%	
Development Rate Detail												
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4							
0	Lab Water Contr	0.995	0.989	0.978	0.994							
0.5		1	1	0.995	0.994							
1		0.995	1	1	1							
2		0.994	0.994	0.989	0.974							
3		0	0	0	0							
4		0	0	0	0							
Development Rate Binomials												
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4							
0	Lab Water Contr	190/191	173/175	175/179	156/157							
0.5		162/162	189/189	195/196	179/180							
1		186/187	185/185	170/170	169/169							
2		176/177	172/173	178/180	152/156							
3		0/116	0/110	0/124	0/130							
4		0/1	0/1	0/1	0/1							

CETIS QC Plot

Report Date: 05 Mar-14 17:20 (1 of 1)

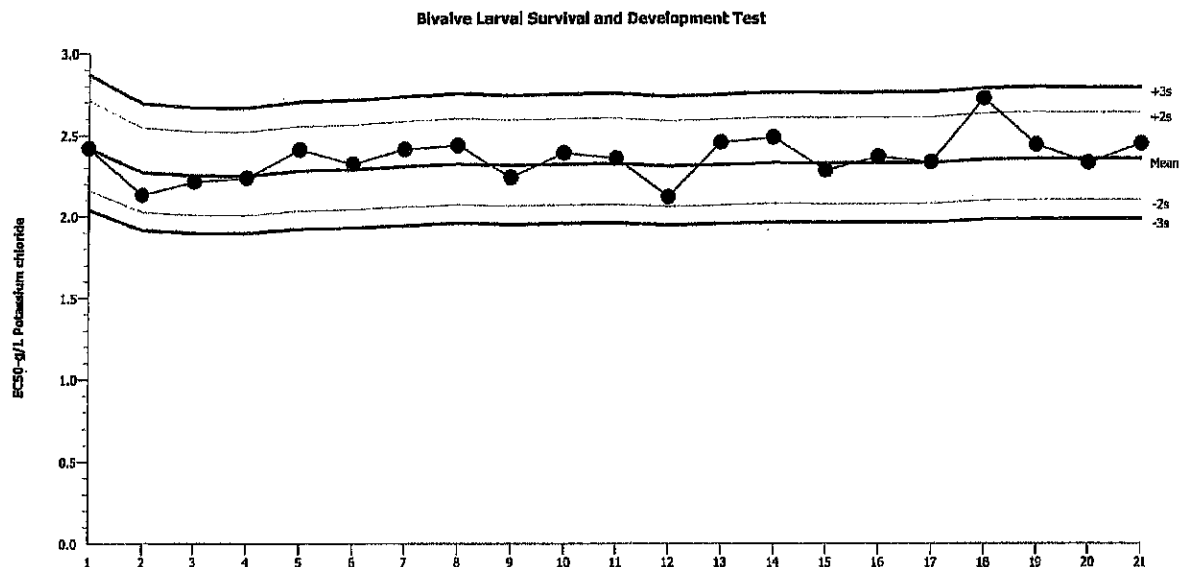
Bivalve Larval Survival and Development Test

Pacific EcoRisk

Test Type: Development-Survival
Protocol: EPA/600/R-95/136 (1995)

Organism: *Mytilus galloprovincialis* (Bay Mussel)
Endpoint: Development Rate

Material: Potassium chloride
Source: Reference Toxicant-REF



Mean: 2.348
Sigma: NA

Count: 20
CV: 5.85%

-2s Warning Limit: 2.095
+2s Warning Limit: 2.631

-3s Action Limit: 1.98
+3s Action Limit: 2.785

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Oct	16	16:17	2.421	0.07313	0.5392			15-4729-5668	09-9006-0766
2			18	15:31	2.13	-0.2176	-1.71			14-9455-9956	00-6656-4134
3			24	13:51	2.213	-0.1349	-1.04			07-7486-5258	12-4981-0284
4			30	15:20	2.234	-0.1135	-0.871			11-2895-1652	02-5267-2624
5		Nov	5	17:33	2.41	0.06215	0.4592			03-5561-9441	20-3893-2621
6			21	15:16	2.32	-0.02835	-0.2135			20-5691-0482	03-4815-2245
7			27	17:45	2.41	0.06196	0.4578			04-6155-9828	05-5569-0544
8		Dec	4	15:44	2.434	0.08623	0.634			14-8498-2872	06-7448-3918
9			12	13:49	2.235	-0.1129	-0.8663			07-3452-0274	04-8262-0765
10			18	14:11	2.387	0.0389	0.2888			04-4375-9945	08-5958-3584
11			27	15:11	2.352	0.004405	0.03295			02-4687-1598	09-0839-1830
12			31	16:25	2.115	-0.2333	-1.84			04-6514-2484	20-3488-8339
13	2014	Jan	9	15:25	2.449	0.1016	0.7449			02-9075-5791	13-0761-7035
14			21	15:46	2.481	0.133	0.9685			03-5782-2218	02-6788-8034
15			23	16:11	2.277	-0.07121	-0.5414			14-7843-2753	11-6356-1634
16			29	16:16	2.362	0.01448	0.1081			04-8512-3256	01-1623-5219
17		Feb	6	17:18	2.329	-0.01913	-0.1438			03-4254-8013	11-4239-7957
18			7	13:47	2.717	0.3694	2.568	(+)		01-9384-3613	14-9880-6070
19			10	16:22	2.434	0.08632	0.6347			17-8218-8470	13-4967-2645
20			12	16:40	2.322	-0.02571	-0.1936			18-3909-2114	01-3442-3701
21			20	14:50	2.441	0.0929	0.6821			06-5660-7780	08-8983-3999

***Mytilus* sp. Development Toxicity Test Count Data**

Client: Reference Toxicant
 Test Material: Potassium Chloride
 Test ID #: 55411
 Project #: 22105

Test Start Date: 2/20/14
 Test End Date: 2/22/14
 Enumeration Date: 2/24/14
 Investigator: AT

Treatment (g/L)	Replicate	Number of Normal Larvae	Number of Abnormal Larvae	Total Number Larvae	Percent Normal Development
Control	A	190	1	191	99.5
	B	173	2	175	98.9
	C	175	4	179	97.8
	D	156	1	157	99.4
0.5	A	162	0	162	100.0
	B	189	0	189	100.0
	C	195	1	196	99.5
	D	179	1	180	99.4
1	A	186	1	187	99.5
	B	185	0	185	100.0
	C	170	0	170	100.0
	D	169	0	169	100.0
2	A	176	1	177	99.4
	B	172	1	173	99.4
	C	178	2	180	98.9
	D	152	4	156	97.4
3	A	0	116	116	0.0
	B	0	110	110	0.0
	C	0	124	124	0.0
	D	0	130	130	0.0
4	A	0	0	0	0.0
	B	0	0	0	0.0
	C	0	0	0	0.0
	D	0	0	0	0.0

Mytilus sp. Development Toxicity Test Water Chemistry Data

Client: _____ Reference Toxicant _____
 Test Material: _____ Potassium Chloride _____
 Test ID#: 55411 Project #: 22105
 Test Date: 2/20/14

Organism Log#: 7960 Age: N/A
 Organism Supplier: Gutofer.
 Control/Diluent: FSW @ 30ppt

Day 0					
Treatment (g/L)	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	18.5	7.80	9.2	29.0	Ref Tex Stock # -
0.5	18.5	7.82	9.3	29.7	Test Solution Prep: SS
1	18.5	7.84	9.3	30.3	New WQ: JLA
2	18.5	7.84	9.4	31.4	Innoculation Date: 2/20/14
3	18.5	7.83	9.4	32.6	Innoculation Time: 1450
4	18.5	7.83	9.4	33.5	Innoculation Signoff: SS
Meter ID	69A	PH19	R507	EC04	

Day 1					
Treatment	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	18.3				Date: 2/21/14
0.5	18.3				WQ: MK
1	18.3				
2	18.3				
3	18.3				
4	18.3				
Meter ID	69A				

Day 2					
Treatment	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	18.2	7.84	7.6	29.4	Termination Date: 2/22/14
0.5	18.2	7.80	7.6	29.9	Termination Time: MK 2 1500
1	18.2	7.82	7.6	30.8	Termination Signoff: MK
2	18.2	7.83	7.7	31.9	Old WQ: D.M.S.
3	18.2	7.84	7.6	32.8	
4	18.2	7.85	7.7	33.9	
Meter ID	69A	PH19	R507	EC04	

Appendix F

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to Topsmelt (*Atherinops affinis*)

CETIS Summary Report

Report Date: 27 Feb-14 13:35 (p 1 of 2)
Test Code: 55117 | 03-4233-7359

Chronic Larval Fish Survival and Growth Test Pacific EcoRisk

Batch ID: 16-1577-6990	Test Type: Growth-Survival (7d)	Analyst: Alison Briden
Start Date: 18 Feb-14 16:15	Protocol: EPA/600/4-91/002 (1994)	Diluent: Laboratory Water
Ending Date: 25 Feb-14 09:25	Species: Atherinops affinis	Brine: Not Applicable
Duration: 6d 17h	Source: Aquatic Biosystems, CO	Age: 12

Sample ID: 17-9785-6733	Code: Eff	Client: Kinnetic Laboratories, Inc
Sample Date: 17 Feb-14 06:00	Material: Effluent	Project: 22017
Receive Date: 18 Feb-14 11:15	Source: Kinnetic Laboratories, Inc.	
Sample Age: 34h (1.7 °C)	Station: Municipality of Anchorage	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
19-5447-8991	7d Survival Rate	2.8	>2.8	NA	NA	35.71	Steel Many-One Rank Sum Test
11-3368-3529	Mean Dry Biomass-mg	2.8	>2.8	NA	24.1%	35.71	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
10-4344-6942	Mean Dry Biomass-mg	IC5	0.0467	0.0179	N/A	2140	Linear Interpolation (ICPIN)
		IC10	0.0935	0.0357	N/A	1070	
		IC15	0.14	0.0536	N/A	713.2	
		IC20	>2.8	N/A	N/A	<35.71	
		IC25	>2.8	N/A	N/A	<35.71	
		IC40	>2.8	N/A	N/A	<35.71	
		IC50	>2.8	N/A	N/A	<35.71	

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	5	1	1	1	1	1	0	0	0.0%	0.0%
0.175		5	1	1	1	1	1	0	0	0.0%	0.0%
0.35		5	1	1	1	1	1	0	0	0.0%	0.0%
0.7		5	1	1	1	1	1	0	0	0.0%	0.0%
1.4		5	1	1	1	1	1	0	0	0.0%	0.0%
2.8		5	1	1	1	1	1	0	0	0.0%	0.0%

Mean Dry Biomass-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	5	1.56	1.42	1.71	1.08	2.08	0.174	0.389	24.8%	0.0%
0.175		5	1.14	1.08	1.2	0.952	1.37	0.0735	0.164	14.4%	27.2%
0.35		5	1.39	1.27	1.5	0.978	1.8	0.138	0.309	22.3%	11.3%
0.7		5	1.28	1.19	1.37	1.09	1.69	0.106	0.237	18.5%	18.1%
1.4		5	1.28	1.22	1.34	1.13	1.45	0.0692	0.155	12.1%	18.3%
2.8		5	1.26	1.2	1.33	1.1	1.53	0.0764	0.171	13.5%	19.2%

CETIS Summary Report

Report Date: 27 Feb-14 13:35 (p 2 of 2)
 Test Code: 55117 | 03-4233-7359

Chronic Larval Fish Survival and Growth Test						Pacific EcoRisk
7d Survival Rate Detail						
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Water Contr	1	1	1	1	1
0.175		1	1	1	1	1
0.35		1	1	1	1	1
0.7		1	1	1	1	1
1.4		1	1	1	1	1
2.8		1	1	1	1	1
Mean Dry Biomass-mg Detail						
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Water Contr	1.08	1.47	2.08	1.39	1.81
0.175		1.02	1.37	1.13	1.22	0.952
0.35		1.8	1.23	1.51	0.978	1.42
0.7		1.23	1.15	1.09	1.25	1.69
1.4		1.15	1.13	1.45	1.23	1.44
2.8		1.16	1.1	1.53	1.21	1.33
7d Survival Rate Binomials						
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Water Contr	5/5	5/5	5/5	5/5	5/5
0.175		5/5	5/5	5/5	5/5	5/5
0.35		5/5	5/5	5/5	5/5	5/5
0.7		5/5	5/5	5/5	5/5	5/5
1.4		5/5	5/5	5/5	5/5	5/5
2.8		5/5	5/5	5/5	5/5	5/5

CETIS Analytical Report

Report Date: 27 Feb-14 13:35 (p 1 of 2)
Test Code: 55117 | 03-4233-7359

Chronic Larval Fish Survival and Growth Test Pacific EcoRisk

Analysis ID: 19-5447-8991 Endpoint: 7d Survival Rate CETIS Version: CETISv1.8.5
Analyzed: 27 Feb-14 13:34 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.8	>2.8	NA	35.71

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Water Control		0.175	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		0.35	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		0.7	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		1.4	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		2.8	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect

ANOVA Table

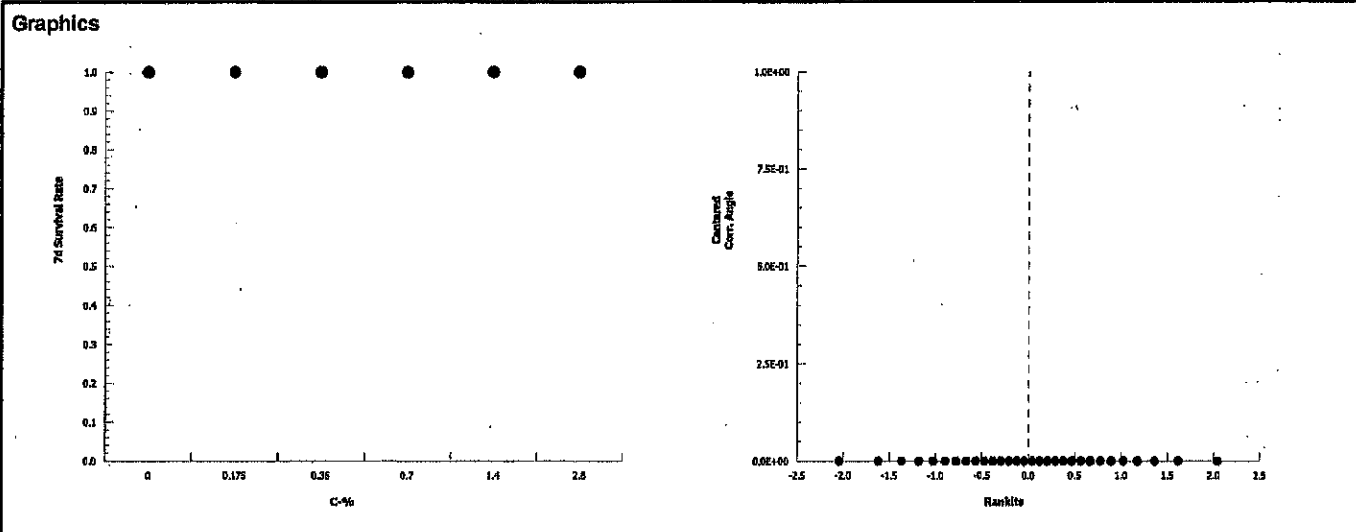
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	5	65500	<0.0001	Significant Effect
Error	0	0	24			
Total	0		29			

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Contr	5	1	1	1	1	1	1	0	0.0%	0.0%
0.175		5	1	1	1	1	1	1	0	0.0%	0.0%
0.35		5	1	1	1	1	1	1	0	0.0%	0.0%
0.7		5	1	1	1	1	1	1	0	0.0%	0.0%
1.4		5	1	1	1	1	1	1	0	0.0%	0.0%
2.8		5	1	1	1	1	1	1	0	0.0%	0.0%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Cont	5	1.35	1.35	1.35	1.35	1.35	1.35	0	0.0%	0.0%
0.175		5	1.35	1.35	1.35	1.35	1.35	1.35	0	0.0%	0.0%
0.35		5	1.35	1.35	1.35	1.35	1.35	1.35	0	0.0%	0.0%
0.7		5	1.35	1.35	1.35	1.35	1.35	1.35	0	0.0%	0.0%
1.4		5	1.35	1.35	1.35	1.35	1.35	1.35	0	0.0%	0.0%
2.8		5	1.35	1.35	1.35	1.35	1.35	1.35	0	0.0%	0.0%



CETIS Analytical Report

Report Date: 27 Feb-14 13:35 (p 2 of 2)
Test Code: 55117 | 03-4233-7359

Chronic Larval Fish Survival and Growth Test Pacific EcoRisk

Analysis ID: 11-3368-3529 Endpoint: Mean Dry Biomass-mg CETIS Version: CETISv1.8.5
Analyzed: 27 Feb-14 13:35 Analysis: Parametric-Control vs Treatments Official Results: Yes

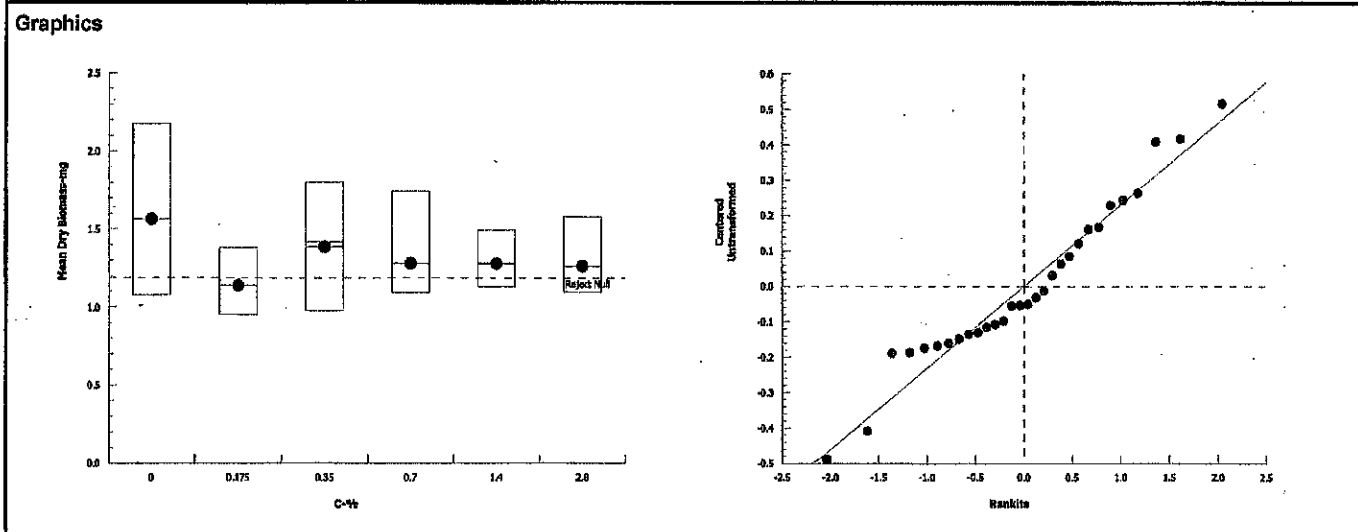
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	24.1%	2.8	>2.8	NA	35.71

Dunnett Multiple Comparison Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Water Control		0.175*	2.66	2.36	0.377	8	0.0269	CDF	Significant Effect
		0.35	1.11	2.36	0.377	8	0.3724	CDF	Non-Significant Effect
		0.7	1.77	2.36	0.377	8	0.1470	CDF	Non-Significant Effect
		1.4	1.79	2.36	0.377	8	0.1434	CDF	Non-Significant Effect
		2.8	1.88	2.36	0.377	8	0.1226	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5163479	0.1032696	5	1.62	0.1935	Non-Significant Effect
Error	1.532115	0.06383813	24			
Total	2.048463		29			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	5.41	15.1	0.3674	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.96	0.903	0.3013	Normal Distribution	

Mean Dry Biomass-mg Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Contr	5	1.56	1.08	2.05	1.47	1.08	2.08	0.174	24.8%	0.0%
0.175		5	1.14	0.935	1.34	1.13	0.952	1.37	0.0735	14.4%	27.2%
0.35		5	1.39	1	1.77	1.42	0.978	1.8	0.138	22.3%	11.3%
0.7		5	1.28	0.986	1.58	1.23	1.09	1.69	0.106	18.5%	18.1%
1.4		5	1.28	1.09	1.47	1.23	1.13	1.45	0.0692	12.1%	18.3%
2.8		5	1.26	1.05	1.48	1.21	1.1	1.53	0.0764	13.5%	19.2%



CETIS Analytical Report

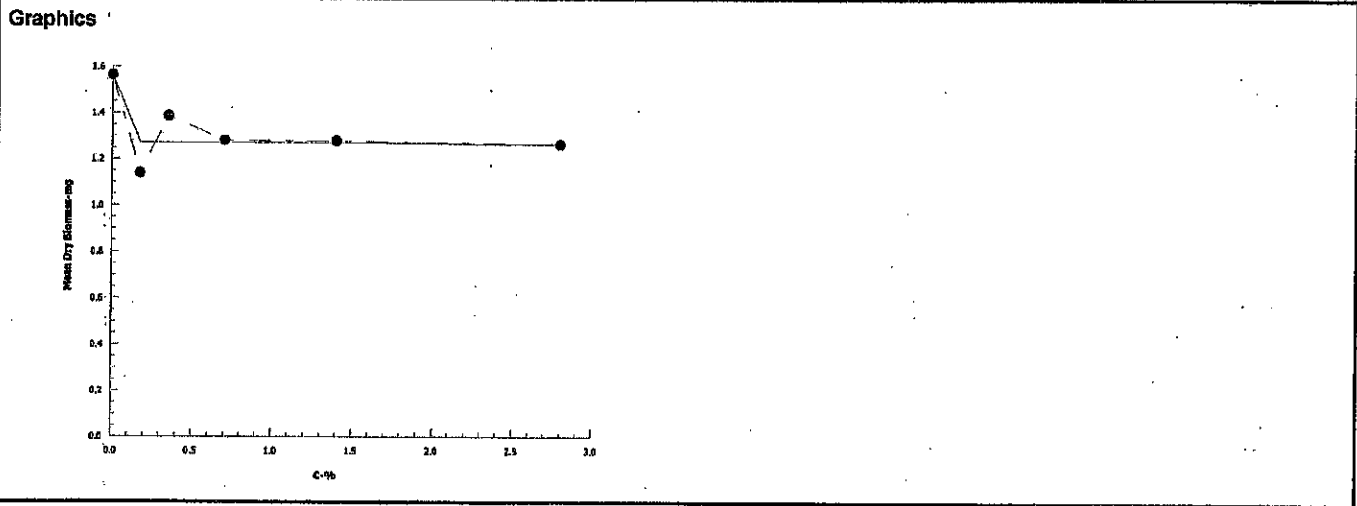
Report Date: 27 Feb-14 13:35 (p 1 of 1)
Test Code: 55117 | 03-4233-7359

Chronic Larval Fish Survival and Growth Test			Pacific EcoRisk		
Analysis ID:	10-4344-6942	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.8.5
Analyzed:	27 Feb-14 13:35	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1741285	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	0.0467	0.0179	N/A	2140	NA	5599
IC10	0.0935	0.0357	N/A	1070	NA	2800
IC15	0.14	0.0536	N/A	713.2	NA	1866
IC20	>2.8	N/A	N/A	<35.71	NA	NA
IC25	>2.8	N/A	N/A	<35.71	NA	NA
IC40	>2.8	N/A	N/A	<35.71	NA	NA
IC50	>2.8	N/A	N/A	<35.71	NA	NA

Mean Dry Biomass-mg Summary			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	5	1.56	1.08	2.08	0.174	0.389	24.8%	0.0%
0.175		5	1.14	0.952	1.37	0.0735	0.164	14.4%	27.2%
0.35		5	1.39	0.978	1.8	0.138	0.309	22.3%	11.3%
0.7		5	1.28	1.09	1.69	0.106	0.237	18.5%	18.1%
1.4		5	1.28	1.13	1.45	0.0692	0.155	12.1%	18.3%
2.8		5	1.26	1.1	1.53	0.0764	0.171	13.5%	19.2%



7 Day Chronic Topsmelt (*A. affinis*) Test DataClient: Kinnetic-AnchorageOrganism Log#: 7945Age: 12 daysTest Material: EffluentOrganism Supplier: ABSTest ID#: 55117 Project #: 22017Control/Diluent: DI + Crystal Sea @ 25 pptTest Date: 2/18/14 Randomization: 5.6.2Control Water Batch: 979

Treatment (% Effluent)	Temp (°C)	pH		D.O. (mg/L)		Salinity (ppt)		# Live Organisms					SIGN-OFF
		new	old	new	old	new	old	A	B	C	D	E	
Control	19.5	7.93		8.0		24.1		5	5	5	5	5	Date: <u>2/18/14</u>
0.175	19.5	7.97		7.9		24.2		5	5	5	5	5	Sample ID #: <u>34100</u>
0.35	19.5	7.97		7.9		24.2		5	5	5	5	5	Test Solution Prep: <u>JE</u>
0.7	19.5	7.97		7.9		24.0		5	5	5	5	5	New WQ: <u>CJD</u>
1.4	19.5	7.96		7.9		24.3		5	5	5	5	5	Initiation Time: <u>1615</u>
2.8	19.5	7.95		7.9		24.4		5	5	5	5	5	Initiation Sign-off: <u>JB</u>
Meter ID:	<u>38A</u>	<u>PH15</u>		<u>RDO4</u>		<u>EC04</u>							
Control	19.4	8.08	7.60	8.0	7.0	24.4	24.1	5	5	5	5	5	Date: <u>2-19-14</u>
0.175	19.4	8.09	7.59	7.9	6.4	24.8	24.3	5	5	5	5	5	Sample ID #: <u>34100</u>
0.35	19.4	8.08	7.59	7.9	6.3	24.9	24.2	5	5	5	5	5	Test Solution Prep: <u>SM</u>
0.7	19.4	8.08	7.60	7.9	6.3	25.1	24.1	5	5	5	5	5	New WQ: <u>JA</u>
1.4	19.4	8.07	7.60	7.9	6.4	25.6	24.0	5	5	5	5	5	Renewal Time: <u>1100</u>
2.8	19.4	8.06	7.59	7.8	6.3	26.8	24.5	5	5	5	5	5	Renewal Sign-off: <u>SIV</u>
Meter ID:	<u>38A</u>	<u>PH15</u>	<u>PH16</u>	<u>RDO5</u>	<u>RDO7</u>	<u>EC08</u>	<u>EC06</u>						Old WQ: <u>JS</u>
Control	19.3	8.13	7.66	8.1	7.0	24.0	23.9	5	5	5	5	5	Date: <u>2/20/14</u>
0.175	19.3	8.15	7.70	8.1	6.9	24.1	24.3	5	5	5	5	5	Sample ID #: <u>34143</u>
0.35	19.3	8.14	7.74	8.1	6.9	24.2	24.3	5	5	5	5	5	Test Solution Prep: <u>CL</u>
0.7	19.3	8.14	7.77	8.1	7.0	24.3	24.5	5	5	5	5	5	New WQ: <u>JA</u>
1.4	19.3	8.13	7.76	8.1	7.0	24.4	25.1	5	5	5	5	5	Renewal Time: <u>1340</u>
2.8	19.3	8.12	7.73	8.1	6.8	24.8	25.7	5	5	5	5	5	Renewal Sign-off: <u>CL</u>
Meter ID:	<u>38A</u>	<u>PH19</u>	<u>PH15</u>	<u>RDO7</u>	<u>RDO5</u>	<u>EC04</u>	<u>EC08</u>						Old WQ: <u>CP</u>
Control	19.4	8.05	7.77	8.2	7.2	24.1	23.9	5	5	5	5	5	Date: <u>2/21/14</u>
0.175	19.4	8.05	7.81	8.2	7.4	24.1	24.3	5	5	5	5	5	Sample ID #: <u>34143</u>
0.35	19.4	8.04	7.81	8.2	7.2	24.1	24.2	5	5	5	5	5	Test Solution Prep: <u>JE</u>
0.7	19.4	8.04	7.81	8.2	7.1	24.2	24.3	5	5	5	5	5	New WQ: <u>MA</u>
1.4	19.4	8.04	7.81	8.2	7.0	24.3	24.6	5	5	5	5	5	Renewal Time: <u>1400</u>
2.8	19.4	8.02	7.78	8.2	7.0	24.5	25.0	5	5	5	5	5	Renewal Sign-off: <u>JE</u>
Meter ID:	<u>38A</u>	<u>PH18</u>	<u>PH16</u>	<u>RDO4</u>	<u>RDO5</u>	<u>EC00</u>	<u>EC04</u>						Old WQ: <u>D.M.S.</u>

7 Day Chronic Topsmelt (*A. affinis*) Test Data

Client: Kinnetic- Anchorage Organism Log#: 7965 Age: 12 days
 Test Material: Effluent Organism Supplier: ABS
 Test ID#: 55117 Project #: 22017 Control/Diluent: DI + Crystal Sea @ 25 ppt
 Test Date: 2/18/14 Randomization: 5.6.2 Control Water Batch: 979

Treatment (% Effluent)	Temp (°C)	pH		D.O. (mg/L)		Salinity (ppt)		# Live Organisms					SIGN-OFF
		new	old	new	old	new	old	A	B	C	D	E	
Control	19.4	7.88	7.81	7.8	7.3	24.0	24.3	5	5	5	5	5	Date: 2/22/14
0.175	19.4	7.89	7.80	7.8	7.2	24.1	24.6	5	5	5	5	5	Sample ID #: 34169
0.35	19.4	7.90	7.76	7.8	7.2	24.1	24.6	5	5	5	5	5	Test Solution Prep: <u>EC</u>
0.7	19.4	7.90	7.78	7.8	7.0	24.2	24.6	5	5	5	5	5	New WQ: <u>ED</u>
1.4	19.4	7.89	7.77	7.8	7.0	24.4	24.8	5	5	5	5	5	Renewal Time: 1600
2.8	19.4	7.88	7.72	7.7	7.0	24.6	25.0	5	5	5	5	5	Renewal-Sign-off: <u>KS</u>
Meter ID: 38A	PH15	PH15	PH15	RD04	RD04	EC06	EC06						Old WQ: <u>mm</u>
Control	19.6	8.30	7.74	7.9	7.3	24.5	24.0	5	5	5	5	5	Date: 2/23/14
0.175	19.6	8.30	7.77	7.9	7.2	24.5	24.2	5	5	5	5	5	Sample ID #: 34169
0.35	19.6	8.29	7.77	7.9	7.1	24.5	24.2	5	5	5	5	5	Test Solution Prep: <u>YS</u>
0.7	19.6	8.29	7.78	7.9	7.1	24.4	24.3	5	5	5	5	5	New WQ: <u>UH</u>
1.4	19.6	8.27	7.76	7.9	7.0	24.2	24.4	5	5	5	5	5	Renewal Time: 1120
2.8	19.6	8.22	7.75	7.9	6.9	24.2	24.7	5	5	5	5	5	Renewal-Sign-off: <u>SM</u>
Meter ID: 38A	PH18	PH15	PH15	RD04	RD07	EC04	EC09						Old WQ: <u>CP</u>
Control	19.4	7.99	7.88	7.7	7.8	24.4	24.4	5	5	5	5	5	Date: 2/24/14
0.175	19.4	8.00	7.84	7.8	7.5	24.6	24.7	5	5	5	5	5	Sample ID #: 34169
0.35	19.4	8.01	7.82	7.8	7.5	24.6	24.7	5	5	5	5	5	Test Solution Prep: <u>SL</u>
0.7	19.4	8.01	7.81	7.8	7.3	24.5	24.6	5	5	5	5	5	New WQ: <u>CP</u>
1.4	19.4	8.00	7.81	7.8	7.3	24.6	24.6	5	5	5	5	5	Renewal Time: 1115
2.8	19.4	8.00	7.80	7.7	7.3	24.7	24.5	5	5	5	5	5	Renewal-Sign-off: <u>SM</u>
Meter ID: 38A	PH15	PH18	PH18	RD07	RD07	EC09	EC09						Old WQ: <u>CD</u>
Control	19.4		7.71		7.0		24.7	5	5	5	5	5	Date: 2/25/14
0.175	19.4		7.72		6.8		25.1	5	5	5	5	5	Termination Time: 0925
0.35	19.4		7.72		6.6		25.0	5	5	5	5	5	Termination Sign-off: <u>SVV</u>
0.7	19.4		7.73		6.6		25.1	5	5	5	5	5	Old WQ: <u>KS</u>
1.4	19.4		7.72		6.7		25.1	5	5	5	5	5	
2.8	19.4		7.66		6.0		25.2	5	5	5	5	5	
Meter ID: 38A			PH16		RD04		EC06						

Chronic Topsmelt Dry Weight and Biomass Data

Client: Kinnetic Anchorage Test ID #: 55117 Project #: 22017
 Test Material: Effluent Tare Weight Date: 2/21/14 Sign-off: AWS
 Test Date: 2/18/14 Final Weight Date: 2/26/14 Sign-off: U

Pan ID	Concentration	Replicate	Initial Pan Weight (mg)	Final Pan Weight (mg)	Initial # of Organisms	Biomass Value (mg)
1	Control	A	171.36	176.74	5	1.08
2		B	178.47	185.75	5	1.47
3		C	173.49	183.89	5	2.08
4		D	161.86	168.81	5	1.39
5		E	165.74	174.78	5	1.81
6	0.175	A	174.40	179.52	5	1.02
7		B	171.70	178.54	5	1.37
8		C	156.92	162.55	5	1.13
9		D	172.54	178.66	5	1.22
10		E	170.66	175.42	5	0.95
11	0.35	A	177.08	186.10	5	1.80
12		B	171.99	178.12	5	1.23
13		C	175.85	183.39	5	1.51
14		D	174.52	179.41	5	0.98
15		E	176.66	183.75	5	1.42
16	0.7	A	176.98	183.11	5	1.23
17		B	165.21	170.94	5	1.15
18		C	166.68	172.14	5	1.09
19		D	160.75	167.00	5	1.25
20		E	161.38	169.83	5	1.69
21	1.4	A	153.78 179.77	185.51	5	1.15
22		B	166.24	171.89	5	1.13
23		C	178.68	185.91	5	1.45
24		D	183.76	189.90	5	1.23
25		E	170.85	178.05	5	1.44
26	2.8	A	174.51	180.29	5	1.16
27		B	181.87	187.35	5	1.10
28		C	184.80	192.44	5	1.53
29		D	172.34	178.39	5	1.21
30		E	170.43	177.07	5	1.33
QA 1			172.93	172.91		—
QA 2			167.94	168.00		—
QA 3			153.78	153.70		—
	Balance ID:		BAL01	BAL01		

Appendix G

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Topsmelt (*Atherinops affinis*)

CETIS Summary Report

Report Date: 10 Mar-14 12:10 (p 1 of 2)
Test Code: 55119 | 19-2394-9241

Chronic Larval Fish Survival and Growth Test Pacific EcoRisk

Batch ID: 01-0181-5328	Test Type: Growth-Survival (7d)	Analyst: Padrick Anderson
Start Date: 18 Feb-14 17:15	Protocol: EPA/821/R/02/014 (2002)	Diluent: Laboratory Water
Ending Date: 25 Feb-14 09:20	Species: Atherinops affinis	Brine: Crystal Sea
Duration: 6d 16h	Source: Aquatic Biosystems, CO	Age: 12

Sample ID: 02-3603-0830	Code: KCI	Client: Reference Toxicant
Sample Date: 18 Feb-14 17:15	Material: Potassium chloride	Project: 22019
Receive Date: 18 Feb-14 17:15	Source: Reference Toxicant	
Sample Age: NA (19.5 °C)	Station: In House	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-7626-4343	7d Survival Rate	0.75	1	0.886	11.8%		Steel Many-One Rank Sum Test
04-5151-6436	Mean Dry Biomass-mg	0.75	>0.75	NA	26.1%		Dunnnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	g/L	95% LCL	95% UCL	TU	Method
18-6425-8168	7d Survival Rate	EC50	0.921	0.881	0.962		Spearman-Kärber
17-0679-8670	Mean Dry Biomass-mg	IC5	0.525	N/A	0.885		Linear Interpolation (ICPIN)
		IC10	0.727	N/A	0.806		
		IC15	0.765	N/A	0.811		
		IC20	0.783	0.422	0.827		
		IC25	0.8	0.552	0.841		
		IC40	0.852	0.743	0.887		
		IC50	0.886	0.792	0.92		

7d Survival Rate Summary

C-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	5	1	1	1	1	1	0	0	0.0%	0.0%
0.25		5	1	1	1	1	1	0	0	0.0%	0.0%
0.5		5	1	1	1	1	1	0	0	0.0%	0.0%
0.75		5	1	1	1	1	1	0	0	0.0%	0.0%
1		5	0.24	0.178	0.302	0	0.4	0.0748	0.167	69.7%	76.0%
1.25		5	0	0	0	0	0	0	0	100.0%	100.0%

Mean Dry Biomass-mg Summary

C-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	5	1.39	1.28	1.51	1.17	1.95	0.143	0.319	22.9%	0.0%
0.25		5	1.38	1.27	1.5	1.04	1.69	0.132	0.296	21.4%	0.68%
0.5		5	1.33	1.28	1.39	1.16	1.49	0.063	0.141	10.6%	4.38%
0.75		5	1.25	1.16	1.34	0.92	1.47	0.108	0.241	19.3%	10.6%
1		5	0.238	0.172	0.304	0	0.472	0.0791	0.177	74.5%	83.0%
1.25		5	0	0	0	0	0	0	0	100.0%	100.0%

CETIS Summary Report

Report Date: 10 Mar-14 12:10 (p 2 of 2)
 Test Code: 55119 | 19-2394-9241

Chronic Larval Fish Survival and Growth Test							Pacific EcoRisk
7d Survival Rate Detail							
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water Contr	1	1	1	1	1	
0.25		1	1	1	1	1	
0.5		1	1	1	1	1	
0.75		1	1	1	1	1	
1		0.4	0.2	0.4	0	0.2	
1.25		0	0	0	0	0	
Mean Dry Biomass-mg Detail							
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water Contr	1.21	1.17	1.31	1.33	1.95	
0.25		1.04	1.29	1.69	1.69	1.21	
0.5		1.16	1.22	1.42	1.49	1.38	
0.75		1.35	1.47	1.43	0.92	1.07	
1		0.32	0.242	0.472	0	0.154	
1.25		0	0	0	0	0	
7d Survival Rate Binomials							
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water Contr	5/5	5/5	5/5	5/5	5/5	
0.25		5/5	5/5	5/5	5/5	5/5	
0.5		5/5	5/5	5/5	5/5	5/5	
0.75		5/5	5/5	5/5	5/5	5/5	
1		2/5	1/5	2/5	0/5	1/5	
1.25		0/5	0/5	0/5	0/5	0/5	

Chronic Larval Fish Survival and Growth Test

Pacific EcoRisk

Test Type: Growth-Survival (7d)

Organism: Atherinops affinis (Topsmelt)

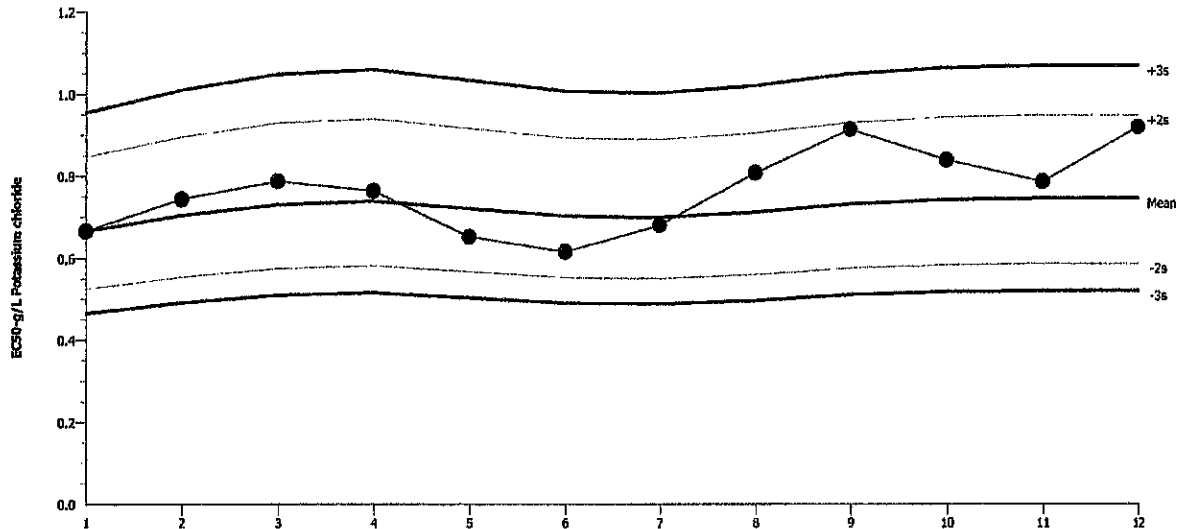
Material: Potassium chloride

Protocol: EPA/821/R/02/014 (2002)

Endpoint: 7d Survival Rate

Source: Reference Toxicant-REF

Chronic Larval Fish Survival and Growth Test



Mean: 0.746

Count: 11

-2s Warning Limit: 0.5869

-3s Action Limit: 0.5205

Sigma: NA

CV: 12.70%

+2s Warning Limit: 0.9484

+3s Action Limit: 1.069

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2008	May	7	16:30	0.6668	-0.07918	-0.935			08-3530-9092	11-7811-2453
2	2010	Apr	6	16:00	0.7443	-0.00170	-0.01906			12-5479-8606	02-7469-6072
3	2011	Dec	1	11:40	0.7882	0.04221	0.4587			13-7822-3920	11-1532-4929
4	2012	Jul	24	15:30	0.7644	0.01842	0.2033			01-2383-8594	00-9503-8608
5		Aug	2	13:25	0.6526	-0.09343	-1.115			20-1020-1892	01-3486-1114
6			4	14:00	0.6157	-0.1303	-1.6			14-2214-8473	08-5995-4246
7	2013	Apr	4	17:45	0.68	-0.06599	-0.7718			20-2186-2907	11-0387-6637
8			5	17:55	0.808	0.06201	0.6654			12-2759-6103	12-6022-6828
9			30	14:05	0.9143	0.1682	1.695			03-6504-2395	09-9900-8353
10		May	2	17:20	0.8393	0.09326	0.9816			04-9429-8023	10-7168-7462
11	2014	Feb	6	16:30	0.7865	0.04048	0.4403			07-2180-3190	15-2982-7918
12			18	17:15	0.9208	0.1748	1.754			19-2394-9241	18-6425-8168

CETIS QC Plot

Report Date: 04 Mar-14 14:37 (1 of 1)

Chronic Larval Fish Survival and Growth Test

Pacific EcoRisk

Test Type: Growth-Survival (7d)

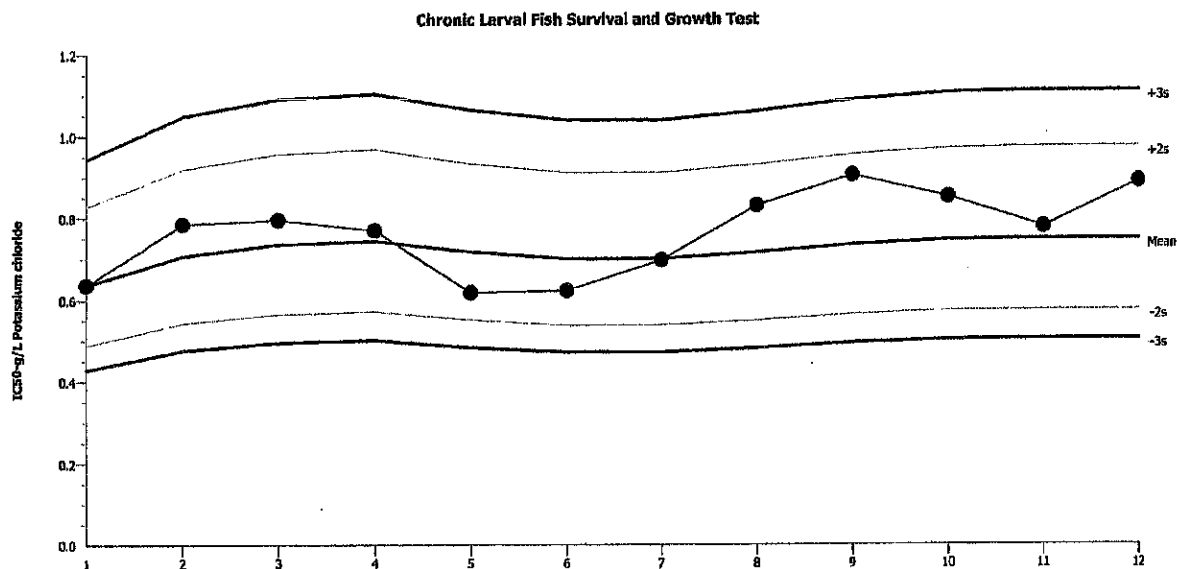
Organism: Atherinops affinis (Topsmelt)

Material: Potassium chloride

Protocol: EPA/821/R/02/014 (2002)

Endpoint: Mean Dry Biomass-mg

Source: Reference Toxicant-REF



Mean: 0.7462

Count: 11

-2s Warning Limit: 0.5736

-3s Action Limit: 0.5029

Sigma: NA

CV: 14.10%

+2s Warning Limit: 0.9706

+3s Action Limit: 1.107

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2008	May	7	16:30	0.6357	-0.1104	-1.218			08-3530-9092	13-4415-4900
2	2010	Apr	6	16:00	0.7843	0.03817	0.3794			12-5479-8606	10-6083-8037
3	2011	Dec	1	11:40	0.795	0.04886	0.4824			13-7822-3920	01-1277-3051
4	2012	Jul	24	16:30	0.7684	0.0222	0.2229			01-2363-8594	12-0055-2382
5		Aug	2	13:25	0.8167	-0.1294	-1.449			20-1020-1892	00-0412-8630
6			4	14:00	0.6213	-0.1249	-1.393			14-2214-8473	05-6270-6827
7	2013	Apr	4	17:45	0.695	-0.05114	-0.54			20-2186-2907	07-3254-0030
8			5	17:55	0.8287	0.08258	0.7982			12-2759-6103	09-6853-5463
9			30	14:05	0.902	0.1558	1.442			03-6504-2395	01-7737-8890
10		May	2	17:20	0.8485	0.1024	0.9775			04-9429-8023	00-1493-5913
11	2014	Feb	6	16:30	0.7757	0.02951	0.2949			07-2180-3190	17-2625-4692
12			18	17:15	0.8862	0.14	1.308			19-2394-9241	14-0082-2050

7 Day Chronic Topsmelt Reference Toxicant Test Data

Client: Reference ToxicantOrganism Log#: 7965 Age: AS 12 daysTest Material: Potassium ChlorideOrganism Supplier: ABSTest ID#: 55119 Project #: 22019Control/Diluent: DI + Crystal Sea @ 25 pptTest Date: 2-18-14 Randomization: 5.6.1Control Water Batch: 979

Treatment (g/L KCl)	Temp (°C)	pH		D.O. (mg/L)		Salinity (ppt)		# Live Organisms					SIGN-OFF
		new	old	new	old	new	old	A	B	C	D	E	
Control	19.5	7.95		7.8		24.0		5	5	5	5	5	Date: <u>2-18-14</u>
0.25	19.5	7.98		7.9		24.4		5	5	5	5	5	RT Stock Batch #: <u>15</u>
0.5	19.5	8.01		8.0		24.7		5	5	5	5	5	Test Solution Prep: <u>SM</u>
0.75	19.5	8.04		8.2		25.2		5	5	5	5	5	New WQ: <u>CJD</u>
1	19.5	8.07		8.1		25.6		5	5	5	5	5	Initiation Time: <u>1715</u>
1.25	19.5	8.09		8.4		25.8		5	5	5	5	5	Initiation Sign-off: <u>AS</u>
Meter ID:	38A	PH15		RD04		EC04							
Control	19.3	8.01	7.72	8.0	7.7	24.1	23.7	5	5	5	5	5	Date: <u>2-19-14</u>
0.25	19.3	8.04	7.80	8.0	7.7	24.2	24.1	5	5	5	5	5	RT Stock Batch #: <u>15</u>
0.5	19.3	8.09	7.85	8.1	7.8	24.6	24.4	5	5	5	5	5	Test Solution Prep: <u>SM</u>
0.75	19.3	8.13	7.76	8.2	7.5	24.9	24.9	5	5	5	5	5	New WQ: <u>CD</u>
1	19.3	8.14	7.74	8.4	7.3	25.3	25.3	4	5	5	2	3	Renewal Time: <u>1100</u>
1.25	19.3	8.17	7.76	8.6	7.2	25.7	25.6	0	0	0	0	0	Renewal-Sign-off: <u>SM</u>
Meter ID:	38A	PH18	PH16	RD07	RD07	EC06	EC09						Old WQ: <u>AS</u>
Control	19.3	8.17	7.72	8.0	7.2	24.0	23.9	5	5	5	5	5	Date: <u>2-20-14</u>
0.25	19.3	8.16	7.75	8.0	7.2	24.3	24.3	5	5	5	5	5	RT Stock Batch #: <u>15</u>
0.5	19.3	8.15	7.77	8.1	7.1	24.6	24.8	5	5	5	5	5	Test Solution Prep: <u>SM</u>
0.75	19.3	8.15	7.81	8.2	7.2	24.9	25.0	5	5	5	5	5	New WQ: <u>SLA</u>
1	19.3	8.15	7.84	8.4	7.2	25.3	25.5	3	3	4	1	2	Renewal Time: <u>1315</u>
1.25	-	-	-	-	-	-	-	-	-	-	-	-	Renewal-Sign-off: <u>Y2</u>
Meter ID:	38A	PH19	PH18	RD07	RD05	EC04	EC08						Old WQ: <u>TM</u>
Control	19.4	8.06	7.90	8.3	7.8	24.0	24.1	5	5	5	5	5	Date: <u>2/21/14</u>
0.25	19.4	8.06	7.88	8.5	7.8	24.2	24.7	5	5	5	5	5	RT Stock Batch #: <u>15</u>
0.5	19.4	8.06	7.87	8.6	7.8	24.6	25.1	5	5	5	5	5	Test Solution Prep: <u>SM</u>
0.75	19.4	8.08	7.85	8.6	7.8	24.9	25.3	5	5	5	5	5	New WQ: <u>D.M.S.</u>
1	19.4	8.09	7.84	9.0	7.7	25.1	25.7	3	1	3	0	2	Renewal Time: <u>1130</u>
1.25	-	-	-	-	-	-	-	-	-	-	-	-	Renewal-Sign-off: <u>Y2</u>
Meter ID:	38A	PH18	PH18	RD04	RD04	EC06	EC06						Old WQ: <u>Y2</u>

7 Day Chronic Topsmelt Reference Toxicant Test Data

Client: Reference Toxicant Organism Log#: 7965 Age: 12 days
 Test Material: Potassium Chloride Organism Supplier: ABS
 Test ID#: 55119 Project #: 22019 Control/Diluent: DI + Crystal Sea @ 25 ppt
 Test Date: 2-18-14 Randomization: 5-6-1 Control Water Batch: 979

Treatment (g/L KCl)	Temp (°C)	pH		D.O. (mg/L)		Salinity (ppt)		# Live Organisms					SIGN-OFF
		new	old	new	old	new	old	A	B	C	D	E	
Control	19.4	8.02	7.42	7.8	7.3	24.1	24.1	5	5	5	5	5	Date: 2/22/14
0.25	19.4	8.05	7.43	7.9	7.3	24.2	24.9	5	5	5	5	5	RT Stock Batch #: 15
0.5	19.4	8.07	7.43	8.2	7.3	24.4	25.2	5	6	5	5	5	Test Solution Prep: SU
0.75	19.4	8.08	7.78	8.5	7.3	24.8	25.6	5	5	5	5	5	New WQ: LH
1	19.4	8.09	7.81	8.6	7.3	25.1	25.8	3	1	2	-	2	Renewal Time: 1030
1.25	-	-	-	-	-	-	-	-	-	-	-	-	Renewal-Sign-off: KP
Meter ID: 38A	PH18	PH15	RDC5	RDO4	ECO9	EC06							Old WQ: CD
Control	19.5	8.16	7.75	7.9	7.1	24.6	24.6	5	5	5	5	5	Date: 2/23/14
0.25	19.5	8.15	7.79	7.9	7.1	24.9	24.6	5	5	5	5	5	RT Stock Batch #: 15/16
0.5	19.5	8.15	7.79	8.1	7.2	25.1	24.9	5	5	5	5	5	Test Solution Prep: CP
0.75	19.5	8.13	7.82	8.2	7.2	25.3	25.2	5	5	5	5	5	New WQ: CP
1	19.5	8.18	7.82	8.2	7.2	25.6	25.5	2	1	2	-	2	Renewal Time: 1110
1.25	-	-	-	-	-	-	-	-	-	-	-	-	Renewal-Sign-off: PR
Meter ID: 38A	PH18	PH15	RDO4	RDO7	ECO4	ECO9							Old WQ: CP
Control	19.5	7.97	7.80	7.9	7.3	24.4	24.8	5	5	5	5	5	Date: 2/24/14
0.25	19.5	8.03	7.83	7.7	7.1	24.5	25.0	5	5	5	5	5	RT Stock Batch #: 16
0.5	19.5	8.07	7.85	7.6	7.1	24.5	25.3	5	5	5	5	5	Test Solution Prep: CP
0.75	19.5	8.10	7.85	7.7	7.0	24.5	25.6	5	5	5	5	5	New WQ: CP
1	19.5	8.14	7.88	7.7	7.0	24.7	25.8	2	1	2	-	2	Renewal Time: 1000
1.25	-	-	-	-	-	-	-	-	-	-	-	-	Renewal-Sign-off: PR
Meter ID: 38A	PH15	PH15	RDO7	RDO7	ECO9	ECO9							Old WQ: CP
Control	19.4		7.70		7.0		25.1	5	5	5	5	5	Date: 2/25/14
0.25	19.4		7.73		7.0		25.4	5	5	5	5	5	Termination Time: 0920
0.5	19.4		7.74		7.0		25.7	5	5	5	5	5	Termination Sign-off: MF
0.75	19.4		7.74		6.9		25.9	5	5	5	5	5	Old WQ: AS
1	19.4		7.79		6.9		26.1	2	1	2	-	1	
1.25	-		-		-		-	-	-	-	-	-	
Meter ID: 38A	PH16				POW4		EC06						

Chronic Topsmelt Dry Weight and Biomass Data

Client: Reference Toxicant Test ID #: 55119 Project #: 22019
 Test Material: Potassium Chloride Tare Weight Date: 2/22/14 Sign-off: AWS
 Test Date: 2/18/14 Final Weight Date: 2/28/14 Sign-off: JLA

Pan ID	Concentration	Replicate	Initial Pan Weight (mg)	Final Pan Weight (mg)	Initial # of Organisms	Biomass Value (mg)
1	Control	A	171.85	177.90	5	1.21
2		B	171.87	177.70	5	1.17
3		C	173.13	179.70	5	1.31
4		D	163.16	169.81	5	1.33
5		E	151.06	160.82	5	1.95
6	0.25 g KCl	A	159.86	165.05	5	1.04
7		B	141.68	148.15	5	1.29
8		C	151.31	159.77	5	1.69
9		D	180.92	189.39	5	1.69
10		E	160.83	166.86	5	1.21
11	0.5 g KCl	A	171.12	176.90	5	1.16
12		B	170.76	176.84	5	1.22
13		C	162.82	169.94	5	1.42
14		D	167.90	175.33	5	1.49
15		E	171.51	178.43	5	1.38
16	0.75 g KCl	A	152.59	159.34	5	1.35
17		B	152.70	160.04	5	1.47
18		C	171.00	178.15	5	1.43
19		D	159.33	163.93	5	0.92
20		E	176.04	181.38	5	1.07
21	1 g KCl	A	154.15	155.75	5	0.32
22		B	161.49	162.70	5	0.242
23		C	171.09	173.45	5	0.472
24		D	156.39	-	5	0
25		E	169.78	170.55	5	0.154
26	1.25 g KCl	A	153.13	-	5	0
27		B	151.54	-	5	0
28		C	152.50	-	5	0
29		D	153.53	-	5	0
30		E	164.32	-	5	0
QA 1			146.90	146.87		-
QA 2			157.48	157.48		-
QA 3			166.92	166.92		-
	Balance ID:		BAL 01	BAL 01		

